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A. D. MELVIN, CHIEF OF BUREAU.

THE NUTRITIVE VALUE OF THE NONPROTEIN OF FEEDING STUFFS.

BY

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LETTER OF TRANSMITTAL.

United States Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., May 8, 1911.

Sir: I have the honor to transmit herewith a manuscript on "The Nutritive Value of the Nonprotein of Feeding Stuffs," by Dr. Henry Prentiss Armsby, who is in charge of the cooperative investigations in animal nutrition by this bureau and the Institute of Animal Nutrition of the Pennsylvania State College. Preliminary to writing a paper dealing with maintenance requirements in feeding animals he has found it necessary to consider the value of the nonprotein nitrogenous substances in the ration. He has therefore in the accompanying manuscript reviewed the literature of investigations on the latter subject and summarized the results, and in conclusion has discussed their bearing. I respectfully recommend the publication of this paper as a bulletin of this bureau.

Respectfully,

A. D. Melvin,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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THE NUTRITIVE VALUE OF THE NONPROTEIN OF FEEDING STUFFS.

INTRODUCTION.

It is well known that the nitrogenous constituents of feeding stuffs comprise, besides the true proteins, numerous other compounds of the most varied nature, including alkaloids, nitrogenous glucosids, amino acids and amids, phosphatids, nitrates, ammonium salts, etc., so that in the aggregate a not inconsiderable proportion of the nitrogen supply of herbivorous animals is derived from these substances. All these diverse nitrogenous compounds have been for convenience grouped under the name "nonprotein." The name, of course, is an abbreviation for nonprotein nitrogenous matters, and, as the foregoing partial enumeration shows, the group is very heterogeneous in its nature.

Alkaloids and nitrogenous glucosids do not appear to be especially abundant in the ordinary feeding stuffs of domestic animals, and where they do occur are distinguished rather by specific physiological effects than by their nutritive value in the ordinary sense. As regards the nutritive value of the phosphatids, comparatively little is known, although it is claimed that the lecithins have a stimulating effect upon growth. Of all the groups of nonnitrogenous substances above enumerated, the amino acids and amids appear to be most abundant. Moreover, they are of special interest because they are products of the protein metabolism of the plant and are to a considerable extent identical with the protein cleavage products which appear to play such a large rôle in animal nutrition. From a practical standpoint, then, the question of the nutritive value of nonproteins is largely identical with that of the nutritive value of the so-called "amids." This question is considered in the present publication solely from the standpoint of their value for the maintenance or production of body protein, without reference to their value as sources of energy.

It has been shown by physiologists that the animal undoubtedly has the power to build up body proteins out of the comparatively simple cleavage products resulting from the digestion of food proteins. It is natural to assume, therefore, that when similar cleavage products

are found in feeding stuffs they are capable of undergoing the same chemical reactions in the body as if they arose from the digestive cleavage of protein. Looking at the question of the nutritive value of the nonprotein from this point of view, it is apparent that the question is to a considerable extent similar to that of the relative values of different proteins. Just as the proportions of the different amino acids, etc., yielded by different proteins vary, so do the proportions of the similar substances found in the nonprotein of different feeding stuffs, while neither the proportions nor the specific compounds are identical in proteins and nonproteins. Evidently, then, it is futile to seek to establish any definite ratio between protein and nonprotein as to their value to the organism, because both of them, but especially the latter, are in this respect more or less variable and indefinite conceptions. The failure to recognize this fact is responsible for not a little of the existing confusion of thought on this question. Thus, many of the earlier investigations of the nutritive value of nonproteins [1] were made upon single amids or amino acids, notably on asparagin, largely because the latter occurs rather abundantly in plants and is readily obtained reasonably pure, although it is not itself a constituent of the protein molecule.[2] In these earlier experiments numerous investigators showed that various single amino acids and amids are katabolized in the animal body, their nitrogen reappearing as urea, although Völtz [3] has lately claimed, contrary to earlier results by Andrlik, Velich, and Stanek,[4] that betain is an exception. As regards their ability to replace the protein of the food, however, or to maintain protein tissue, these experiments indicated a marked apparent difference between carnivorous and omnivorous animals on the one hand and the herbivora, particularly ruminants, on the other.

EXPERIMENTS ON CARNIVORA.

The earlier experiments made upon carnivora as a rule failed to show that amids could to any degree serve to protect the protein tissue of the body from katabolism. More recent experiments have, upon the whole, confirmed these results. While numerous investigations have shown beyond a doubt that the body has the power to build up protein from the mixture of at least very simple cleavage products obtained by prolonged enzym hydrolysis, or even by acid hydrolysis,[5] experiments in which single amino acids or even three or

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[1] Compare Armsby, Henry P. The Principles of Animal Nutrition. Third edition, revised, New York, 1908, pp. 52–58.

[2] Asparagin is the amid of aspartic acid, which is one of the cleavage products of all proteins thus far investigated.

[3] Völtz, W. Untersuchungen über die Verwertung des Betains durch den Wiederkäuer (Schaf). Archiv für die gesammte Physiologie, Band 116, Heft, 5–6, pp. 307–333. Bonn, 1907.

[4] Zeitschrift Zuckerindustrie in Böhmen, Band 27, p. 14.

[5] Abderhalden, Emil, and Frank, Oskar. Weiterer Beitrag zur Frage nach der Verwertung von tief abgebautem Eiweiss im tierischen Organismus. XII Mitteilung. Zeitschrift für physiologische Chemie. Band 64, Heft 2–3, pp. 158–163. Strassburg, 1910.

four, or the mixture contained in vegetable extracts, have been fed have failed to establish satisfactorily the ability of the organism to form protein from them. The principal investigations on this question have been by Völtz, Lehmann, Rosenfeld, and Müller.

Völtz[1] has reported three series of experiments upon dogs. In the first he compared the effect of adding to the basal ration consumed by a mature dog, on the one hand, 1 gram of nitrogen in the form of protein of various kinds and on the other hand one-half gram in the form of protein and the remainder as asparagin. As regards the relative values of the proteins, the experiments are open to the criticism that the protein content of the basal ration was too high. The latter contained 0.37 to 0.75 gram total nitrogen per kilogram live weight, as compared with the 0.2 to 0.3 gram per kilogram which apparently suffices for maintenance.[2] In other words, surplus protein was being katabolized in the body in these experiments. It is not surprising to find, therefore, that the effects upon the nitrogen balance of adding more protein to the ration were irregular and difficult to interpret. The asparagin, however, in every case was found to be inferior to protein in its power of maintaining or increasing the body protein. It may be noted also in passing that the asparagin increased the nitrogen content of the feces. If we may judge from the results upon herbivora, to be considered subsequently, this may be ascribed to an increase in the so-called metabolic products, especially mucus and epithelial detritus.

In the second series of experiments, also on a mature dog, a basal ration containing about 0.75 gram nitrogen per kilogram body weight was fed in the first and last periods. In the remaining periods 1 gram of nitrogen in various forms (asparagin, ammonium acetate, acetamid, and glycocol) was added to the basal ration. Völtz bases his conclusions upon the average of the last 7 days of the 10-day periods. His results show during the periods of nonprotein feeding an excess of nitrogen in the urine over the average of the basal period greater than the amount of nonprotein nitrogen added to the ration. That is, the nonproteins diminished the gain of nitrogen by the body. In his final table Völtz assumes the fecal nitrogen for each period as equal to that of the first, disregarding an observed steady increase

[1] Völtz, W. Über den Einfluss verschiedener Eiweisskörper und einiger Derivate derselben auf den Stickstoffumsatz, mit besonderer Berücksichtigung des Asparagins. Archiv für die gesammte Physiologie, Band 107, Heft 7–9, pp. 360–414. Bonn, 1905.

Über den Einfluss des Lezithins auf den Eiweissumsatz ohne gleichzeitige Asparaginzufuhr und bei Gegenwart dieses Amids. Zeitschrift für die gesammte Physiologie, Band 107, Heft 7–9, pp. 415–425. Bonn, 1905.

Über das Verhalten einiger Amidsubstanzen allein und im Gemisch im Stoffwechsel der Karnivoren, Zeitschrift für die gesammte Physiologie, Band 112, Heft 7–8, pp. 413–438. Bonn, 1906.

Völtz, W., and Yakuwa, G. Über die Verwertung verschiedener Amidsubstanzen durch Carnivoren. Zeitchrift für die gesammte Physiologie, Band 121, Heft 3–4, pp. 117–149. Bonn, 1908.

[2] Compare Armsby, Principles of Animal Nutrition, p. 143, 1908; and Chittenden, The Nutrition of Man, Chapter VII, 1907.

in the subsequent periods, including the last basal ration. In this manner he computes that in period 6, in which a mixture of the nonproteins used in the preceding periods was fed, the nitrogen katabolism remained practically the same as in the basal period, and hence concludes that the mixture was more effective in this respect than its ingredients separately, and argues that experiments upon a single amid or amino acid are inconclusive as regards the value of the mixed nonproteins of natural products. The general correctness of this point of view was pointed out in the introduction, but Kellner[1] has called attention to the rather remarkable nature of Völtz's calculations, and has shown that when the actual analytical results are made the basis of the calculation it appears that the animal, which in the first period was gaining nitrogen, was steadily approaching a condition of nitrogen equilibrium in the last period, and that the addition of the nonproteins to the ration produced no distinct effect.

In his third series of experiments, upon one growing and two mature dogs, Völtz followed the same general plan as in the previous series, but used five-day periods, alternating with similar periods on the basal ration. As before, 1 gram of nitrogen in the same four forms was added to a basal ration containing, in the case of the mature animals, 0.58 to 0.73 gram, and in that of the growing animals from 0.43 to 0.46 gram nitrogen per kilogram body weight; that is, materially more than the minimum protein requirement. In these experiments, contrary to the earlier ones, the amount of nitrogen contained in the feces was slightly less instead of greater in the periods in which the nonproteins were fed. The results are corrected for the effect of the nonproteins upon the nitrogen excretion at the beginning of the following basal period from the data for the daily nitrogen excretion. Thus corrected, the periods in which acetamid, ammonium acetate, and a mixture of nonproteins were consumed showed a considerable retention of nonprotein nitrogen, while the periods with asparagin and with glycocol failed to do so. It may be noted that dog No. 3 (mature) showed a distinct tendency toward a gain of nitrogen even on the basal ration, while dog No. 2 (immature) did not.

Rosenfeld,[2] at the suggestion of C. Lehmann, investigated the influence of the bulk of food upon the utilization of asparagin by the dog by cooking very finely ground hay with the remaining feed. He found in the hay periods a considerable retention of the nitrogen of the asparagin added to the ration, while when albumin was substituted for asparagin the retention was less. He concludes that

[1] Kellner, O. Zur Kenntniss der Wirkung nicht eiweissartiger Stickstoffverbindungen auf den Stickstoffumsatz im Tierkörper. Archiv für die gesammte Physiologie, Band 113, Heft 7–8, pp. 480–486. Bonn, 1906. See p. 484.

[2] Cited by Völtz, Archiv für die gesammte Physiologie, Band 107, Heft 7–9, p. 365.

under these conditions there is either an action of the ferment organisms similar to that occurring in herbivora[1] or that some substances resorbed from the hay facilitate the utilization of the asparagin.

Lehmann[2] reports experiments performed by Rosenfeld on another phase of the same general idea, viz, that the rate of resorption may materially affect the nutritive value of nonprotein. He points out that in experiments upon carnivorous animals soluble nonproteins (usually asparagin) have commonly been given in a single dose so that they were rapidly resorbed, while with herbivorous animals, on the contrary, the resorption from ordinary feeding stuffs would be relatively slower. In the first case, therefore, the system would be more or less flooded temporarily with the nonprotein, which would presumably be subject to rapid nitrogen cleavage; and Lehmann cites Graffenberger's experiments[3] in illustration of this effect. To test the truth of this view, Lehmann prepared a mixture of asparagin and a solution of celluloid and allowed it to dry out in small grains. This coated asparagin was compared with untreated material and with blood albumin, it being shown by preliminary trials that the solution of the asparagin was rendered less rapid by the treatment, but that it was completely digested by the animal. The subject, a dog, received a basal ration, consisting of meat, rice, lard, and ash ingredients, which contained 0.55 gram nitrogen per kilogram of live weight. No statement of the energy content of the ration is made, but the fact that the nitrogen katabolism seems to have steadily increased in the basal periods suggests an insufficient supply. Increasing amounts of nitrogen in the three forms mentioned were added in successive three-day periods in amounts ranging from 1 to 2 grams, a period upon a basal ration preceding each of the three series of trials.

In summarizing his results Lehmann compares for each three-day period the excess of nitrogen digested over that digested in the period immediately preceding with the excess of nitrogen found in the urine of the same three days. Compared in this way he finds that the free asparagin increased slightly the loss of nitrogen from the body, while the coated asparagin maintained nitrogen equilibrium and the blood albumin caused a slight gain. Neglecting the minute amounts of nitrogen given off in the hair, the results may also be summarized as in the following table:

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[1] Compare p. 13.

[2] Lehmann, C. Beiträge zur Kenntniss der Wirkung des Asparagins auf den Stickstoffumsatz im Thierkörper. Archiv für die gesammte Physiologie, Band 112, Heft 7–8, pp. 339–351. Bonn, 1906.

[3] Graffenberger, L. Versuche zur Feststellung des zeitlichen Ablaufes der Zersetzung von Fibrin, Leim, Pepton und Asparagin im menschlichen Organismus. Zeitschrift für Biologie, Band 28, pp. 318–344. München and Leipzig, 1891.

Average nitrogen per day—Lehmann's experiments.

Items.	Digested.	In urine.	Gain or loss.
	Grams.	*Grams.*	*Grams.*
Basal period	5.13	4.99	+0.14
Asparagin, coated	6.55	6.53	+0.02
Basal period	5.11	5.10	+0.01
Asparagin, free	6.66	6.85	−0.19
Basal period	5.11	5.19	−0.08
Blood albumin	6.43	6.40	+0.03
Basal period	5.14	5.28	−0.14

It appears from these results that the asparagin had a tendency to increase the breaking down of nitrogenous body material, but the differences hardly seem very significant. Kellner [1] has criticized Lehmann's conclusions because he failed to take due account of the lag in the excretion of urinary nitrogen and of the gradual increase in the nitrogen katabolism upon the basal ration. He shows from Lehmann's data that some of the nitrogen added to the basal ration appeared in the urine on the first day or two of the following basal period (these days are not included in the data of the table). Recalculating the results from this point of view, Kellner computes that both forms of asparagin had an equal effect in materially increasing the nitrogen katabolism, while the albumin, on the other hand, diminished it somewhat. In reply Lehmann [2] denies that Kellner is justified in assuming an increasing nitrogen katabolism for the basal periods and thus estimating what the nitrogen balance would have been in each period without the added nitrogen. By comparing the actual figures Lehmann shows a distinct negative balance upon the free asparagin as compared with a slight positive balance on the coated asparagin and the albumin.

Müller [3] has repeated Lehmann's experiments with coated and uncoated asparagin, with the difference that he added more nitrogen to the basal ration in proportion to the live weight than did Lehmann and also added sufficient nonnitrogenous material to compensate for the difference in energy between asparagin and an amount of albumin containing the same quantity of nitrogen. On the basal periods the ration contained 0.56 to 0.64 gram nitrogen per kilogram live weight, and the animal was quite exactly in nitrogen equilibrium. After correcting the observed results in each period for the influence of the lag of nitrogen excretion as shown by the succeeding basal period he

[1] Kellner, O. Zur Kenntnis der Wirkung nicht eiweissartiger Stickstoffverbindungen auf den Stickstoffumsatz im Tierkörper. Archiv für die gesammte Physiologie, Band 113, Heft 7–8, pp. 480–486. See p. 484. Bonn, 1906.

[2] Lehmann, C. Nochmals zur Wirkung des Asparagins auf den Stickstoffumsatz im Tierkörper. Archiv für die gesammte Physiologie, Band 115, Heft, 115, pp. 448–451. Bonn, 1906.

[3] Müller, Max. Weitere Untersuchungen über die Wirkung des Asparagins auf den Stickstoffumsatz und Ansatz des Tierkörpers. Archiv für die gesammte Physiologie, Band 117, Heft 10–12, pp. 497–537. Bonn, 1907.

finds some retention of the added nitrogen in all cases, but notably less in the case of the free asparagin. Including a correction for the small gain or loss in the basal ration, he computes a retention of the following amounts of nitrogen per day:

	Gram.
Asparagin, coated	0.35
Asparagin, uncoated	.18
Blood albumin	.35
Dextrin	.06

In a subsequent investigation [1] Müller has compared the effect of blood albumin with that of the mixed nitrogenous material contained in an aqueous extract of hay. A mature dog received a basal ration containing 0.55 gram nitrogen per kilogram live weight. To this were added in subsequent periods equal amounts of nitrogen in the forms of blood albumin and of hay extract, no addition of nonnitrogenous material being made and no correction being made for the lag in the nitrogen excretion. A small plus balance of nitrogen was observed in both cases, but it was much smaller with the hay extract than with the blood albumin, being, indeed, almost or quite negligible.

Müller's experiments have also been criticized by Kellner [2] on the ground that in the second series the nitrogen lag was not taken account of, while he suspects analytical errors in the nitrogen determinations of both series (although this is denied by Müller [3]), while Friedländer [4] regards the differences observed by Müller as within the limits of analytical error and also criticizes his short periods.

Quite aside, however, from the points raised by Kellner and others, there is one feature of all these experiments which renders their results inconclusive, viz, the fact that, as already pointed out, the nitrogenous substances to be tested were *added* to a basal ration which already contained a surplus of protein over the maintenance requirement. When the animal was in nitrogen equilibrium with the basal ration, therefore, the nitrogenous cleavage products arising from the digestion of the protein were doubtless being deamidized to a considerable extent and their nitrogen excreted as urea. Such being the case, while additional protein might cause more or less gain of nitrogen for a time, additional nonprotein might easily produce indirectly a similar effect, without implying any formation of protein from it, simply by taking the place of some of the cleavage

[1] Müller, Max. Untersuchungen über die Nährwirkung im Heu enthaltener Nichteiweisse. Journal für Landwirthschaft, Band 55, pp. 123–141. Berlin, 1907.

[2] Kellner, O. Notiz betreffend die Nährwirkung des Asparagins. Archiv für die gesammte Physiologie, Band 118, Heft 11–12, pp. 641–642. Bonn, 1907.

Kellner, O. Untersuchungen über die Nährwirkung der im Heu enhaltenen nichteiweissartigen Stickstoffverbindungen. Journal für Landwirtschaft, Band 56, pp. 49–52. Berlin, 1908.

[3] Müller, Max. Richtigstellung der von O. Kellner gemachten kritischen Bemerkungen. Journal für Landwirtschaft, Jahrgang 56, pp. 193–194. Berlin, 1908.

[4] Friedländer, Konrad. Zur Frage des Eiweissersatzes durch Amide. Die landwirtschaftlichen Versuchs-Stationen, Band 67, pp. 283–312. Berlin, 1907. See pp. 289–292.

products whose nitrogen was previously split off. In other words, it is possible that the nitrogen stored up in the body had its origin in the surplus protein of the food and not in the nonprotein added. The fact that the retention of nitrogen when nonprotein was fed was frequently less than that when a corresponding amount of protein was given may possibly be explained by the fact that one or two single amino acids could not fully replace in this respect the mixture arising from the digestion of the protein of the food. Taking all these points into consideration, we seem warranted in concluding that it has not yet been satisfactorily shown that carnivorous animals can produce body protein either from one or a few of the amino acids or from the mixture of nonproteins contained in the vegetable products thus far investigated.

EXPERIMENTS ON OMNIVORA.

To a number of earlier investigations on omnivorous animals, mostly rats and mice, may be added more recent experiments by Henriques and Hansen [1] upon rats, directed, like the earlier ones, to a somewhat different aspect of the problem than that studied in the foregoing experiments on carnivora. They investigated the question whether asparagin or the mixture of nonprotein nitrogenous materials found in various vegetable substances (potatoes, roots, and seedlings of beans and barley), when constituting the sole source of protein, were capable of maintaining the protein tissue of the body. Their results fully confirmed the earlier ones of Politis and of Gabriel and showed that under these circumstances the nonprotein can not perform the functions of protein. A continuous loss of nitrogen from the body was observed, which was substantially at the same rate as when only nonnitrogenous nutrients were consumed.

EXPERIMENTS ON HERBIVORA.

But while there is no satisfactory evidence that either single nonproteins or the mixtures of them found in vegetable products are available to either carnivora or omnivora as a source of protein, with herbivora, as previously indicated, the case is different.[2] With the latter class of animals a considerable number of experiments are on record, of which Weiske's are the earliest, which have shown that asparagin added to a ration poor in protein is able partially to replace the latter. Zuntz appears to have been the first to advance the idea that this marked difference between the two classes of animals might be due to the difference in their digestive processes and particularly

[1] Henriques, V., and Hansen, C. Über die Bedeutung der sogenannten Pflanzenamide für den Stickstoffumsatz im tierischen Organismus. Zeitschrift für physiologische Chemie, Band 54, pp. 169-187. Strassburg, 1907-8.

[2] Compare Armsby, Principles of Animal Nutrition, pp. 53-58. New York, 1908.

to the great development of organized ferments in the digestive tract of herbivora. He suggests that soluble nonprotein introduced into the digestive canal of herbivora may be used as nitrogenous food by the organisms in preference to the less soluble proteins, particularly in the first stomach of ruminants, before the digestion of the proteins begins, so that the latter are to a greater or less extent protected from destruction, while it is possible that the nonproteins are thus synthesized to protein by the organisms and later digested by the animal.

The validity of this suggestion was confirmed by Kellner [1] in experiments upon lambs, in which the gain of nitrogen by the animal upon a ration poor in protein was very notably increased by the addition to the ration not only of asparagin, but also of ammonium acetate, which it has not been supposed that the body tissues can synthesize to protein. He showed also that this effect was accompanied by an increased digestibility of the crude fiber and nitrogen-free extract of the ration, presumably due to the greater activity of the micro-organisms under the influence of the soluble nitrogenous food. Tryniszewsky also observed a similar effect upon the digestibility of the nonnitrogenous ingredients of the feed, although the effect upon the nitrogen balance was less decided.

This view, which has been generally accepted, regards the value of the nonprotein in the feed of herbivora as indirect, due to its protecting protein from destruction. The multiplication of organisms under the influence of an increased supply of nonprotein nitrogen has, however, another aspect, as appears when we inquire what becomes of the nitrogen which they assimilate. Presumably it becomes part of the protoplasm of the organisms and in this way may produce one of two effects. If any of the nitrogen of the feces has its origin in the nonprotein of the feed—that is, if the bacterial protein formed from the latter is indigestible—it is necessary to modify considerably the ordinary interpretation of digestion experiments upon rations containing nonproteins. Hitherto the latter, being soluble in water, have been regarded as totally digestible. If, however, part of the fecal nitrogen is derived from them, the digestion experiment as ordinarily computed gives too high a result for the resorbed nonprotein and correspondingly too low a result for the resorbed protein, and this leads to ascribing to the former nutritive effects really due to the latter.

On the other hand, if the bacterial protein formed from the nonproteins is digestible, we have in the activity of these organisms in

[1] Kellner, O. Untersuchungen über den Einfluss des Asparagins und Ammoniaks auf den Eiweissumsatz der Wiederkäuer. Zeitschrift für Biologie, Band 39, pp. 313–376. München und Leipzig, 1900.

the digestive tract a means of converting nonprotein into available protein, and so virtually adding to the protein supply in the food by a sort of symbiosis. It may be remarked that the presence of bacteria in the feces does not necessarily disprove this, since the latter may readily come from the lower part of the alimentary canal.

Since the interpretation of the results of feeding experiments upon herbivora must be materially affected by the question of the fate of the nonprotein in the digestive tract, it seems necessary to consider this aspect of the question first.

BEHAVIOR OF NONPROTEINS IN DIGESTIVE TRACT OF HERBIVORA.

There are a considerable number of experiments on record in which the protein of the feces has been distinctly increased by feeding materials containing much nonprotein nitrogen, and this has been interpreted as indicating the formation by bacteria in the digestive tract of indigestible nitrogenous compounds. In other cases, however, scarcely any such effect has been observed.

In the early experiments of Weiske, as well as in the later ones of Chomsky, more or less increase in the total nitrogen of the feces was observed to result when asparagin was fed. In Kellner's experiments upon lambs, just referred to, asparagin was substituted for an equal weight of starch in the first, third, and fourth series, while in the second and third periods of the second series ammonium acetate and asparagin, respectively, were added to the basal ration. The total excretion of fecal nitrogen was as follows:

Excretion of fecal nitrogen—Kellner's experiments.

Items.	Lamb I.	Lamb II.
	Grams.	*Grams.*
Series I:		
Period 1, basal ration	6.51	6.32
Period 2, asparagin substituted	5.54	5.77
Series II:		
Period 1, basal ration	6.09	6.17
Period 2, ammonium acetate added	6.63	6.16
Period 3, asparagin added	6.25	5.63
Series III:		
Period 1, basal ration	7.17	6.95
Period 2, asparagin substituted	7.08	6.85
Series IV:		
Period 1, basal ration	7.66	6.09
Period 2, asparagin substituted	7.70	6.28
Period 3, basal ration	7.14	5.87
Average:		
Basal ration	6.79	6.51
Asparagin	6.64	6.13
Ammonium acetate	6.63	6.16

The experiments of Tryniszewsky also showed substantially the same result, the crude protein of the feces being in period 2, basal ration, 206 grams; period 3, asparagin, 210 grams; period 4, basal ration, 191 grams.

Neither of the foregoing experiments shows very distinct evidence of any increase of the nitrogenous matter of the feces as a result of feeding asparagin or ammonium acetate. It must be remembered, however, that the feces contain nonprotein nitrogen in the form of metabolic products. It is possible, therefore, that the protein nitrogen might have increased in these experiments even though the total nitrogen did not. In more recent experiments, therefore, the comparison has been made upon the protein nitrogen of the feces—that is, the nitrogen which is either insoluble in water or precipitable by copper hydrate. Experiments by Andrlik, Velich, and Stanek,[1] in which glutaminic and aspartic acids were added to the basal ration of a young wether, yielded the following results, which fail to show any material influence of the added nonprotein upon the fecal nitrogen. An earlier series [2] likewise showed no increase in the total nitrogen of the feces as a result of adding betain to the basal ration.

Results of Andrlik, Velich, and Stanek's experiments.

Items.	Feed.		Feces.	
	Protein nitrogen.	Nonprotein nitrogen.	Protein nitrogen.	Nonprotein nitrogen.
	Grams.	*Grams.*	*Grams.*	*Grams.*
I. Basal ration	8.42	1.190	4.067	.390
II. Glutaminic acid	8.52	3.072	4.443	.450
III. Basal ration	8.63	1.190	4.350	.350
IV. Aspartic acid	8.63	3.296	3.990	.410
V. Basal ration	8.58	1.190	4.425	.350
Average on basal ration	8.39	1.190	4.281	.363
Average on nonprotein ration	8.58	3.184	4.216	.430

The experiments upon which special stress has been laid, however, are those of Völtz [3] and of Friedländer.[4]

Völtz fed to a sheep a ration consisting of straw, potatoes, and molasses, or the distiller's residues from the latter—that is, a ration poor in protein and rich in nonprotein. The results, so far as they bear upon the particular point under discussion, are shown in the following table, from which it appears that without exception more protein was found in the feces than in the feed.

[1] Andrlik, K., and Velich, K. Ueber die bedeutung der Glutamin und Asparaginsäure als Nahrstoffe Zeitschrift für Zuckerindustrie in Böhmen, Jahrgang 32, Heft 6, pp. 313–342. Prag, 1908.

[2] Velich, Alois, and Stanek, Vladimir. Ueber das Betain in physiologischchemischer Beziehung. Zweiter Bericht. Zeitschrift für Zuckerindustrie in Böhmen, Jahrgang 29, Heft 4, pp. 205–219. Prag, 1905.

[3] Völtz, W. Ueber die Verwertung des Amidgemisches der Melasse durch den Widerkäuer. Archiv für gesammte Physiologie, Band 117, Heft 10–12, pp. 541–563. Bonn, 1907.

[4] Friedländer, Konrad. Zur Frage des Eiweissersatzes durch Amide. Die Landwirtschaftlichen Versuchs-Stationen, Band 67, pp. 283–312. Berlin, 1907.

Protein nitrogen in feed and feces—Völtz's experiments.

Periods.	Daily feed.				Protein nitrogen per day.	
	Straw.	Potatoes.	Molasses.	Distiller's residue from molasses.	In feed.	In feces.
	Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*
I	500.0	500	....	200	3.42	4.41
II	498.4	500	400	....	3.42	3.92
III	497.9	500	400	....	3.42	3.43
IV	498.4	....	600	....	2.96	3.61
V	394.0	....	560	....	2.42	2.98

In Friedländer's experiments also two sheep received rations poor in protein but containing considerable nonprotein in the form of beet molasses. In two periods asparagin was added to this ration and in one a form of commercial protein. The results are shown in the following table, from which it appears that in this case, too, the protein nitrogen of the feces was in excess of that in the feed, except in the period in which commercial protein was fed.

Protein nitrogen in feed and feces—Friedländer's experiments.

Periods.	Rations.				Protein nitrogen.			
					Sheep I.		Sheep II.	
	Hay.	Molasses-peat.	Asparagin.	Protein.	In feed.	In feces.	In feed.	In feces.
	Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*
I	200	625	....	....	3.32	4.01	3.32	4.09
II	200	730	....	....	3.45	5.56	3.43	5.34
III	200	730	30	....	3.16	4.84	....	....
IV	200	625	....	52	10.56	4.35	10.56	4.21
V	200	625	30	....	3.32	4.44	3.32	4.18

Both these experiments have been interpreted as showing a formation of indigestible protein from the nonprotein of the feed.

Just [1] experimented upon two growing lambs through 10 periods. In the first and last periods a basal ration consisting of hay, starch, and sugar was fed. In the intermediate periods nitrogenous matter was added in various forms, while an attempt was made to keep the so-called starch values of the rations unchanged by diminishing the starch and sugar, although this object was not entirely attained. In the following table is shown for each period the difference as regards nonprotein nitrogen and protein nitrogen between the feed and the feces of the period and the average of the two basal rations.

[1] Just, Jaroslav. Vergleichende Untersuchungen über die Wirkungen des Eiweisses und einiger nichteiweissartiger Stickstoffverbindungen auf den Fleischansatz beim Wiederkäuer. Die landwirtschaftlichen Versuch-Stationen, Band 69, pp. 393-460. Berlin, 1908.

Protein and nonprotein nitrogen in feed and feces—Just's experiments.

	Feed.		Feces—Lamb I.		Feces—Lamb II.	
	Nonprotein nitrogen.	Protein nitrogen.	Nonprotein nitrogen.	Protein nitrogen.	Nonprotein nitrogen.	Protein nitrogen.
	Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*
Period 2 (molasses)	2.62	10.61	0.95	7.00	1.22	7.12
Basal	1.36	7.95	.76	5.94	0.75	5.88
	1.26	2.66	.19	1.06	.47	1.24
Period 3 (gluten)	[1] 1.52	[1] 11.17	1.03	6.00	.90	6.21
Basal	1.36	7.95	.76	5.94	.75	5.88
	.16	3.22	.27	.06	.15	.33
Period 4 (malt sprouts extract)	3.53	9.10	1.42	7.07	1.48	6.76
Basal	1.36	7.95	.76	5.94	.75	5.88
	2.17	1.15	.66	1.13	.73	.88
Period 5 (gluten)	1.48	10.51	.89	5.96	.83	5.99
Basal	1.36	7.95	.76	5.94	.75	5.88
	.12	2.56	.13	.02	.08	.11
Period 6 (potato "flocken")	3.24	10.67	1.16	6.11	.97	6.42
Basal	1.36	7.95	.76	5.94	.75	5.88
	1.88	2.72	.40	.17	.22	.54
Period 7 (gluten)	1.53	11.82	.82	6.36	.69	6.13
Basal	1.36	7.95	.76	5.94	.75	5.88
	.17	3.87	.06	.42	−.06	.25
Period 8 (grass extract)	3.35	8.14	.43	6.33	.43	6.35
Basal	1.36	7.95	.76	5.94	.75	5.88
	1.99	.19	−.33	.39	−.32	.47
Period 9 (gluten)	1.51	11.10	.58	6.08	.51	6.32
Basal	1.36	7.95	.76	5.94	.75	5.88
	.15	3.15	−.18	.14	−.24	.44

[1] For Lamb III, 1.49 and 10.77, respectively.

Summarizing these differences, we find that the average increase of protein nitrogen of the feces over that present in the basal periods was as shown in the following table:

Increases in protein nitrogen of feces—Just's experiments.

Items.	Lamb I.	Lamb II.
	Grams.	*Grams.*
Average of gluten	0.16	0.28
Molasses	1.06	1.24
Malt sprouts extract	1.13	.88
Potato "flocken"	.17	.54
Grass extract	.39	.47

While Just's results afford no instance in which the protein nitrogen of the feces exceeds that of the feed, they show a marked effect of the extracts and especially of the molasses in increasing the former.

Kellner and his associates [1] have recently reported the results of two series of experiments upon growing lambs, using a ration composed of oat straw, starch, and sugar; that is, one containing very little protein and practically no nonprotein. To this mixture there was added in the first period ammonium acetate and asparagin and in the second period wheat gluten containing a slightly smaller amount of nitrogen, the energy value of the ration being kept the same by a reduction in the amount of starch. In the second series of experiments, a third period was also added in which ammonium acetate and asparagin were added to the ration of the second period. The results, so far as they relate to the question under discussion, are as shown in the following table.

Although in period 1 of each series the protein nitrogen of the feces is greater than that of the feed, a comparison with period 2 shows that this is not due to any materially greater excretion of protein nitrogen when the ammonium acetate was fed—the amounts being sensibly the same—but to the very small amount of true protein contained in the ration.

Protein and nonprotein nitrogen in feed and feces—Kellner's experiments.

Items.	In feed.		In feces.	
	Nonprotein nitrogen.	Protein nitrogen.	Nonprotein nitrogen.	Protein nitrogen.
Series I:	*Grams.*	*Grams.*	*Grams.*	*Grams.*
Lamb I. Period 1	11.01	1.18	2.53	4.20
Period 2	.92	10.19	.67	4.25
Series II:				
Lamb II. Period 1	14.27	1.55	.71	4.37
Period 2	1.12	11.74	1.44	4.50
Period 3	14.89	11.62	1.03	4.48
Lamb III. Period 1	14.27	1.55	1.76	4.92
Period 2	1.12	11.72	1.86	4.33
Period 3	14.89	11.62	1.24	4.73

The foregoing results make it clear that there is a marked difference between different forms of nonprotein as regards their effect upon the excretion of protein nitrogen in the feces. While Kellner's results (both the earlier and later ones) and those of Tryniszewski show no increase as the result of the addition or substitution of ammonium salts or asparagin, those of Völtz, Friedländer, and Just show a marked increase from the use of plant extracts, especially molasses. Moreover, Just's results show striking differences between the various materials which are by no means related to the content of nonprotein, as is evident from the following table, which shows the increase in the nonprotein nitrogen of the basal ration caused by the

[1] Kellner, O., Eisenkolbe, P., Flebbe, R., and Neumann, R. Untersuchungen über den Einfluss einiger nicht-eiweissartiger Stickstoffverbindungen auf den Eiweissumsatz beim Wiederkäuer. Die landwirtschaftlichen Versuch-Stationen, Band 72, pp. 437-458. Berlin, 1910.

addition of the materials named, and the average increase of the protein nitrogen in the feces.

Comparative increase of nonprotein nitrogen in feed and feces—Just's experiments.

Items.	Nonprotein nitrogen added to basal ration.	Increase of protein nitrogen in feces.
	Grams.	*Grams.*
Gluten	0.17	0.22
Molasses	1.26	1.15
Malt sprouts extract	2.17	1.00
Potato "flocken"	1.88	.36
Grass extract	1.99	.43

These general results are abundantly confirmed by those obtained by Morgen and his associates in the course of their extensive investigations upon the nutritive value of nonproteins for milk production. The results of these experiments will be stated in greater detail immediately in discussing another phase of the subject. In a considerable number of these experiments they consistently observed no increase in the protein nitrogen of the feces to result from the substitution or addition of ammonium salts or asparagin, while, on the other hand, plant extracts had a marked but variable effect in this direction.

These well-established facts are scarcely consistent with the hypothesis of the formation of indigestible bacterial protein from the nonprotein of the feed. If such a formation takes place, it is difficult to see why it should not be quite as marked in the case of readily soluble and assimilable nitrogenous substances like ammonium salts as in that of plant extracts, nor why the effect in the latter case should not be more or less proportional to the amount of nonprotein present.

Moreover, Morgen [1] has shown that plant extracts containing relatively little nonprotein nitrogen may also cause an increase of the protein nitrogen of the feces. He compared extracts prepared from grass and from dried sugar-beet pulp, containing in the dry matter—

	Dried beet-pulp extract.	Grass extract.
	Per cent.	*Per cent.*
Protein	2.84	9.45
Nonprotein	.97	4.20
	3.81	13.65

[1] Morgen, A., Beger, C., and Westhausser, F. Untersuchungen über die Verwertung der Ammonsalze und der nicht-eiweissartigen Stickstoffverbindungen der Futtermittel für die Lebenserhaltung und Milchbildung, sowie über die Frage, ob aus diesen Stoffen unverdauliches Eiweiss gebildet wird. Die landwirtschaftlichen Versuch-Stationen, Band 73, pp. 285–396. Berlin, 1910. See pp. 320 and 350.

In experiments in which these extracts were added to a basal ration, equivalent amounts of starch and sugar were withdrawn, and likewise an amount of protein equal to the true protein of the extracts, disregarding the nonprotein. Upon the average of two animals, the protein and the nonprotein nitrogen of the feed and the protein nitrogen of the feces were as shown in the following table. While the extract of dried sugar-beet pulp increased the nonprotein of the feed somewhat, the increase of protein nitrogen in the feces is relatively much greater than that caused by a much larger increase of nonprotein in the grass-extract ration.

Comparison of extracts of dried beet pulp and of grass—Morgen's experiments.

Items.	In feed.		In feces.
	Protein nitrogen.	Nonprotein nitrogen.	Protein nitrogen.
	Grams.	*Grams.*	*Grams.*
Basal ration	18.32	0.68	5.39
Extract of dried beet pulp	18.61	1.03	6.11
Grass extract	19.35	2.33	7.43

Still further, Morgen [1] found that in numerous digestion experiments upon straw—that is, a feed containing practically no nonprotein—the nitrogen of the feces exceeded that of the feed in 15 cases out of 18. This was by no means a new observation, there being, as Morgen points out, numerous experiments on record showing an apparent negative digestibility of the nitrogen in straw and similar feeding stuffs poor in protein.

The obvious explanation of this phenomenon in the case of the straw is the presence of the so-called nitrogenous metabolic products in the feces. Their presence and their influence upon the apparent digestibility of the food were early recognized, but the first attempt at a quantitative determination of their amount in the excreta of herbivorous animals is due to Kellner,[2] who estimated the average amount of metabolic nitrogen in the feces at 0.4 gram per 100 grams organic matter digested. Subsequent investigations by Pfeiffer [3] and by G. Kühn [4] have resulted in the development of a method by which

[1] Loc. cit., Band 73, p. 337.

[2] Kellner, O. Beiträge zur quantitativen Bestimmung des verdauten Proteins. Biedermann's Centralblatt für Agrikulturchemie, Jahrgang 9, pp. 107–110. Leipzig, 1880. Untersuchungen über Protein verdanung. Biedermann's Centralblatt für Agrikulturchemie, Jahrgang 9, pp. 763–765. Leipzig, 1880.

[3] Pfeiffer, Th. Beiträge zur Frage über die Bestimmung der Stoffwechselproducte im tierischen Koth. Journal für Landwirtschaft, Jahrgang 33, pp. 149–192. Berlin, 1885.

Pfeiffer, Th. Die Verdaulichkeit getrockneter Rübenschnitzel, sowie die Bestimmung der Verdauungscoëfficienten stickstoffhaltiger Futterbestandtheile im allgemeinen. Journal für Landwirtschaft, Jahrgang 34, pp. 425–453. Berlin, 1886.

Pfeiffer, Th. Die Bestimmung des Stickstoffs der Stoffwechselproducte. Zeitschrift für physiologische Chemie, Band 10, pp. 561–576. Strassburg, 1886.

[4] Kühn, Gustav, Thomas, A., Böttcher, O., Köhler, A., Zielstorff, W., and Barnstein, A. Untersuchungen über die Verdauung stickstoffhaltiger Futterbestandteile durch Behandlung mit Magen- und Pankreas-Extrakten. Die landwirtschaftlichen Versuchs-Stationen, Band, 44. pp. 188–256. Berlin, 1894

the amount of metabolic nitrogen contained in the feces can be at least approximately determined. The method is based upon the process of artificial digestion with pepsin or pepsin and trypsin as early proposed by Stöckhardt and by Hofmeister and worked out later by Stutzer [1] for the laboratory determination of the digestibility of feeds. A comparison of Stutzer's method with natural digestion early showed a lower result in the latter case, especially on coarse fodders and those poor in nitrogen; and since it is difficult to see how the action of the digestive juices could be less effective than that of the same enzyms in the laboratory, this led to the conclusion that the difference was due to metabolic nitrogen. To test this, Pfeiffer fed 2 pigs a nitrogen-free ration of starch, sugar, oil, paper pulp, and ash ingredients, to which in a subsequent period pure digestible protein (conglutin) was added. From the first experiment the feces contained an average of 0.4 gram nitrogen per 100 grams of digested dry matter, which must have been in the form of metabolic products, since the feed contained no nitrogen. In the second, in which the protein was assumed to be entirely digestible, practically the same result (0.39 gram metabolic nitrogen) was obtained, showing that the result of the first experiment was not rendered abnormal by the lack of nitrogen in the feed.

For the present purpose, the most interesting feature of the investigation is that these metabolic nitrogenous materials in the fresh dung are soluble in pepsin hydrochloric acid, and can thus be removed from the feces, leaving a residue insoluble in pepsin which represents substantially the indigestible nitrogenous matter of the feed. Kellner [2] also reports similar unpublished results upon a sheep receiving nonnitrogenous matter.[3] That this is actually the case is shown by comparisons of the pepsin-insoluble nitrogen in the feces with that contained in the feed, such as have been made by Pfeiffer and by Kühn. The latter in particular has shown that by certain modifications of Stutzer's method very close agreement can be obtained between artificial and natural digestion if the comparison in the latter case be made upon the pepsin-insoluble nitrogen of the feces. In other words, the pepsin-insoluble nitrogen of the feed reappears quantitatively in the feces.

If, however, indigestible bacterial protein is formed from the nonprotein of the feed, this process should increase the pepsin-insoluble nitrogen of the feces, while if the observed increase in the fecal nitrogen in some cases is due to metabolic products these should

[1] Stutzer, A. Die Einwirkung von saurem Magensaft auf die stickstoffhaltigan Bestandtheile der Mohnkuchen. Journal für Landwirtschaft, Jahrgang 28, pp. 195–208. Berlin, 1881.

Stutzer, A. Beiträge zur Werthbestimmung der Futtermittel. Journal für Landwirtschaft, Jahrgang 28, pp. 435–453. Berlin, 1881.

[2] Die Ernährung der Landwirtschaftliche Nutztiere, 5th ed., p. 32.

[3] It is, of course, conceivable that the feces may contain food nitrogen which was potentially soluble but which has escaped solution, but it hardly seems likely that any such nitrogen would be dissolved by further treatment with pepsin in the laboratory.

be soluble in pepsin. That is, we may regard the pepsin-insoluble nitrogen of the feces as representing indigestible feed protein, and the pepsin-soluble nitrogen as contained in metabolic products, part of which are protein (mucus, epithelium, etc.) and part nonprotein (residues of digestive fluids, etc.). A comparison of the pepsin-insoluble nitrogen in the feed and feces, therefore, affords the best available means of determining whether the ingestion of nonprotein has resulted in the formation of indigestible bacterial protein. Such comparisons have been made in the investigations by Morgen et al., already referred to.

The results of the experiments in 1907 [1] include digestion experiments on 3 ewes in milk and a partial report of experiments upon 2 milch goats. The basis of the ration consisted of dried sugar beet pulp, straw, straw pulp, and a small amount of hay, and contained very little nonprotein. To this basal ration there were added wheat gluten, starch, oil, and sugar. In the experiments upon sheep a portion of the protein supplied by the wheat gluten was replaced in certain periods by nonprotein contained in an extract prepared from malt sprouts. Owing to the rather unpalatable nature of the rations, only about three-fourths of the ration of the protein periods was consumed in the nonprotein periods. In the experiments with goats the malt sprouts extract seems to have been simply added to the basal ration. The results of these experiments, so far as they relate to the question under discussion, are contained in the following table:

Forms of nitrogen in feed and feces—Morgen's experiments of 1907.

Items.	In feed.			In feces.		
	Nonprotein nitrogen.	Pepsin-soluble protein nitrogen.	Pepsin-insoluble nitrogen.	Nonprotein nitrogen.	Pepsin-soluble protein nitrogen.	Pepsin-insoluble nitrogen.
Sheep 13:	*Grams.*	*Grams.*		*Grams.*	*Grams.*	*Grams.*
Period 1, protein	0.81	21.46		0.96	5.24	3.50
Period 2, nonprotein	5.60	11.14		2.47	5.36	3.39
Period 2–period 1	+4.79	−10.32		+1.51	+ .12	− .11
Sheep 25:						
Period 1, protein	.69	17.55		1.18	3.95	3.23
Period 3, nonprotein	3.58	10.21		1.77	3.86	3.07
Period 3–period 1	+2.89	− 7.34		+ .59	− .09	− .16
Sheep 30:						
Period 1, protein	.69	17.55		.93	4.78	2.93
Period 3, nonprotein	1.30	9.15		1.13	2.84	2.87
Period 3–period 1	+ .61	− 8.40		+ .20	−1.94	− .06
Goat 28:						
Periods 1 and 5, basal	.48	11.70		1.43	3.26	2.45
Period 2, nonprotein	5.97	12.05		2.94	5.09	3.96
Period 2–periods 1 and 5	5.49	.35		1.51	1.83	1.51

[1] Morgen, A., Beger, C., and Westhausser, F. Weitere Untersuchungen über den Einfluss der nichteiweissartigen Stickstoffverbindungen der Futtermittel auf die Milchproduktion. Die landwirtschaftlichen Versuchs-Stationen, Band 68, pp. 333–432. Berlin, 1908.

Forms of nitrogen in feed and feces—Morgen's experiments of 1907—Continued.

Items.	In feed.			In feces.		
	Nonprotein nitrogen.	Pepsin-soluble protein nitrogen.	Pepsin-insoluble nitrogen.	Nonprotein nitrogen.	Pepsin-soluble protein nitrogen.	Pepsin-insoluble nitrogen.
Goat 28—Continued.	*Grams.*	*Grams.*		*Grams.*	*Grams.*	*Grams.*
Periods 1 and 5, basal	0.48	11.70		1.43	3.26	2.45
Period 4b, protein	.55	17.39		1.96	3.03	3.38
Period 4b–periods 1 and 5	.07	5.69		.53	− .23	.93
Goat 39:						
Period 1, basal	.40	9.50		1.05	2.11	2.41
Period 3a, nonprotein	5.96	9.86		2.34	4.02	3.26
Period 3a–period 1	5.56	.36		.69	1.91	.85

It appears from this table that with the goats the increase of the nonprotein nitrogen caused a distinct increase in the fecal nitrogen, and that of this increase one-fourth to one-third was insoluble in pepsin. Since, however, the pepsin-insoluble nitrogen of the feed was not determined, it is possible that part of this increase may have arisen from a greater amount of the latter in the feed; but obviously only a small part of the increase in the feces can thus be accounted for, since the entire increase of the protein nitrogen of the feed was only 0.36 gram, as compared with 0.85 gram and 1.51 grams in the feces. On the other hand, however, it is to be noted that there appears to have been a similar increase of the pepsin-insoluble nitrogen in Period IVb, in which wheat gluten instead of malt sprouts extract was added to the basal ration.

In the experiments with sheep the difference in the total amount of the ration noted above gives rise to some difficulty in interpreting the results. It appears clear from the table, however, that the pepsin-insoluble nitrogen of the feces was practically unaffected by the substitution of nonprotein for protein.

In the investigations of 1908,[1] digestion experiments were made on 7 ewes in milk, on 2 milking goats, and on 6 wethers, the pepsin-insoluble nitrogen of the feeding stuffs being determined. The experiments on the milk animals were of the same general nature as those of 1907 and the results were similar. Relatively more hay was fed than in the previous year, making the rations more palatable, and they were eaten without residue throughout. A considerable variety of nonprotein-containing materials were used, viz, extracts of malts prouts, grass, and mangels; ammonium acetate, tartarate, and

[1] Morgen, A., Berger, C., and Westhausser, F. Weitere Untersuchungen über die Verwertung der nicht-eiweissartigen Stickstoffverbindungen der Futtermittel sowie der Ammonsalze durch das milchgebende Tier, unter besonderer Berücksichtigung der stickstoffhaltigen Stoffwechselprodukte. Die landwirtschaftlichen Versuch-Stationen, Band 71, pp. 1–170. Berlin, 1909.

phosphate, and asparagin. Each nonprotein period was interpolated between two protein periods, the nonprotein (or in two cases carbohydrates) being substituted for protein. The following table gives the average results obtained for each form of nonprotein compared with the average of the results with the same animals for the protein rations.

Forms of nitrogen in feed and feces—Morgen's experiments of 1908.

Items.	In feed.			In feces.		
	Nonprotein nitrogen.	Protein nitrogen.		Nonprotein nitrogen.	Protein nitrogen.	
		Pepsin-soluble.	Pepsin-insoluble.		Pepsin-soluble.	Pepsin-insoluble.
	Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*
Ammonium salts:						
Protein rations (16 periods)	0.58	14.77	2.55	0.77	3.17	2.91
Ammonium rations (9 periods)	6.23	8.93	2.33	.86	2.82	2.78
	+5.65	− 5.84	− .22	+ .09	− .35	− .13
Asparagin:						
Protein rations (6 periods)	.56	14.26	2.48	.73	3.18	2.84
Asparagin rations (2 periods)	6.19	8.41	2.25	.91	2.57	2.56
	+5.63	− 5.85	− .23	+ .18	− .61	− .28
Carbohydrates:						
Protein rations (4 periods)	.60	15.45	2.66	.92	2.91	2.76
Carbohydrate rations (2 periods)	.51	9.32	2.48	.65	3.41	2.73
	− .09	− 6.13	− .18	− .27	+ .50	− .03
Malt sprouts extract:						
Protein rations (4 periods)	.64	16.55	2.86	.68	3.31	3.11
Extract rations (2 periods)	7.98	10.91	3.52	2.35	5.82	4.69
	+7.34	− 5.64	+ .66	+1.67	+2.51	+1.58
Grass extract:						
Protein rations (3 periods)	.59	15.10	2.60	.84	3.29	2.97
Extract rations (1 period)	4.32	11.14	3.63	1.03	4.26	5.59
	+3.73	− 3.96	+1.03	+ .19	+ .97	+2.62
Mangel extract:						
Protein rations (2 periods)	.56	14.26	2.48	.84	2.63	2.84
Extract rations (1 period)	4.13	11.08	3.42	1.05	3.24	4.38
	+3.57	− 3.18	+ .94	+ .21	+ .61	+1.54

The experiments with ammonium salts and asparagin and also the substitution of carbohydrates for protein resulted in a very slight decrease of the pepsin-insoluble nitrogen in the feces, which was closely paralleled by a similar decrease in the feed. In other words, these nonprotein materials showed practically no effect upon the fecal nitrogen. The extract experiments, on the other hand, as in the previous year's tests, showed a marked increase in the pepsin-insoluble nitrogen of the feces, of which increase, however, from 40 to 60 per cent is accounted for by the increase in the pepsin-insoluble nitrogen of the feed. The extracts appear, therefore, to have had a distinct effect in increasing the pepsin-insoluble nitrogen of the feces.

This increase, however, is much less than that of the total protein nitrogen, especially in the case of the malt sprouts extract.

The digestion experiments on the wethers in this year were intended to test the question whether the low apparent digestibility of the protein of the malt sprouts extract as computed from the foregoing digestion experiments was due to the formation of indigestible bacterial protein, or to the so-called depression of digestion due to the small amount of protein in the rations. To test this question the animals received in the first period only straw and malt sprouts extract, while in period 2 wheat gluten was added to this mixture. The average result for the four animals was as follows:

Forms of nitrogen in feed and feces—Morgen's experiments of 1908.

Items.	In feed.			In feces.		
	Non-protein nitrogen.	Protein nitrogen.		Non-protein nitrogen.	Protein nitrogen.	
		Pepsin-soluble.	Pepsin-insoluble.		Pepsin-soluble.	Pepsin-insoluble.
	Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*
Period 1, straw and extract	4.68	6.28	1.93	1.33	2.98	2.99
Period 2, same plus gluten	4.73	11.05	2.20	1.55	2.85	2.91
Difference	−.05	−4.77	−.27	−.22	+.13	+.08

As compared with what may be assumed to be the normal results in period 2, the straw and extract ration of period 1, containing much less protein, showed nevertheless on the average a small increase in the protein nitrogen of the feces, which, however, was large enough to be significant in only two out of the four animals (sheep F and G). This increase in the pepsin-soluble portion of the fecal nitrogen may be taken to indicate that some of the food protein escaped digestion; that is, we have here the so-called depression of digestibility due to a relative excess of nonnitrogenous nutrients. The results computed in this way correspond with the digestion coefficients as computed by Morgen.

In a third period with two of the wethers the malt sprouts extract was replaced by starch, sugar, and gluten, the amount of the latter being made approximately equal to the true protein of the malt extract; while in a fourth period, with only one animal, wheat gluten equivalent to the total nitrogen of the malt sprouts extract was given. The results were as follows:

Forms of nitrogen in feed and feces—Morgen's experiments of 1908.

Items.	In feed.			In feces.		
	Nonprotein nitrogen.	Protein nitrogen.		Nonprotein nitrogen.	Protein nitrogen.	
		Pepsin-soluble.	Pepsin-insoluble.		Pepsin-soluble.	Pepsin-insoluble
Sheep C:	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*
Period 1	4.80	6.60	2.18	1.24	2.63	3.41
Period 3	.05	6.29	1.74	.34	1.81	2.38
Period 1-period 3	4.75	.31	.44	.90	.82	1.03
Sheep F:						
Period 1	4.80	6.21	1.45	1.05	2.79	2.68
Period 3	.05	5.51	1.34	.42	1.49	1.65
Period 1-period 3	4.75	.64	.11	.63	1.30	1.03
Sheep T:						
Period 1	4.80	6.21	1.45	1.05	2.79	2.68
Period 4	.13	10.31	1.53	.22	1.36	2.12
Period 1-period 4	4.67	−4.10	−.08	.83	1.43	.56

We see here the same effect as before of the malt sprouts extract in increasing the total protein nitrogen of the feces and likewise to a less extent the pepsin-insoluble portion of it.

The experiments upon milking animals in 1909[1] were made chiefly with ammonium acetate, which was both substituted for protein in the basal ration and also added to the latter. Similar addition experiments were also made with grass extract and with the extract of dried sugar-beet pulp containing relatively much less nonprotein. The results upon the different animals were, on the whole, very uniform, and the averages of the following table appear sufficient to demonstrate the teaching of the experiments:

Forms of nitrogen in feed and feces—Morgen's experiments of 1909.

Items.	In feed.			In feces.		
	Nonprotein nitrogen.	Protein nitrogen.		Nonprotein nitrogen.	Protein nitrogen.	
		Pepsin-soluble.	Pepsin-insoluble.		Pepsin-soluble.	Pepsin-insoluble.
Ammonium acetate added to basal ration (7 trials):	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*
Acetate ration	10.73	15.72	2.93	1.10	3.16	2.81
Basal ration	.69	15.72	2.93	1.06	3.24	2.83
Difference	10.04	.00	.00	.04	−.08	−.02

[1] Morgen, A., Beger, C., and Westhausser, F. Untersuchungen über die Verwertung der Ammonsalze und der nicht-eiweissartigen Stickstoffverbindungen der Futtermittel für die Lebenserhaltung und Milchbildung, sowie über die Frage, ob aus diesen Stoffen unverdauliches Eiweiss gebildet wird. Die landwirtschaftlichen Versuchs-Stationen, Band 73, Heft 4–5, pp. 285–396. Berlin, 1910.

Forms of nitrogen in feed and feces—Morgen's experiments of 1909—Continued.

Items.	In feed.			In feces.		
	Nonprotein nitrogen.	Protein nitrogen.		Nonprotein nitrogen.	Protein nitrogen.	
		Pepsin-soluble.	Pepsin-insoluble.		Pepsin-soluble.	Pepsin-insoluble.
Ammonium acetate substituted in basal ration (4 trials):	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*
Acetate ration	11.00	6.14	2.58	1.15	3.08	2.88
Basal ration	.73	16.41	3.06	1.06	3.23	2.96
Difference	10.27	−10.27	−.48	.09	−.15	−.08
Grass extract added to basal ration (2 trials):						
Extract ration	2.33	15.82	3.53	1.09	3.25	4.18
Basal ration	.68	15.44	2.88	.61	2.81	2.59
Difference	1.65	.38	.65	.48	.44	1.59
Dried beet-pulp extract added to basal ration (2 trials):						
Extract ration	1.03	15.38	3.23	.95	2.62	3.49
Basal ration	.68	15.44	2.88	.61	2.81	2.59
Difference	.35	−.06	.35	.34	−.19	.90

It is clear that the ammonium salts produced practically no effect upon the amount of fecal nitrogen excreted in any form. The grass extract, on the other hand, shows the same effect as in previous experiments, viz, an increase of both the protein nitrogen and the pepsin-insoluble nitrogen of the feces, the latter being greater than can be accounted for by the corresponding increase in the feed. On the other hand, as already noted, the beet-pulp extract, containing much less nonprotein nitrogen, produced a relatively greater effect in this direction, particularly as regards the pepsin-insoluble nitrogen, indicating that the result is not caused by the nonprotein.

The digestion experiments of 1909 were made upon 4 wethers with the same general plan and object as in 1908, viz, to test the hypothesis of the formation of indigestible bacterial protein from the nonprotein. In these experiments wheat gluten was added to a basal ration containing little protein and much nonprotein. They differed from the experiments of the previous year in the fact that the nonprotein consisted of ammonium acetate, which, as the results on milking animals just cited show, does not produce indigestible bacterial protein. The basal ration of period 1 consisted of straw and ammonium acetate. The average results for all 4 animals were as shown in the following table:

Forms of nitrogen in feed and feces—Morgen's experiments of 1909.

Periods.	In feed.			In feces.		
	Nonprotein nitrogen.	Protein nitrogen.		Nonprotein nitrogen.	Protein nitrogen.	
		Pepsin-soluble.	Pepsin-insoluble.		Pepsin-soluble.	Pepsin-insoluble.
	Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*
1	5.54	1.41	1.50	0.26	1.43	1.78
2	5.57	3.02	1.57	.10	1.48	1.78
3	5.66	7.53	1.79	1.17	.58	1.65

As in the previous experiments, the amount of pepsin-insoluble nitrogen in the feces was practically unaffected. On the other hand, the protein nitrogen of the feces as ordinarily computed was much less in amount in period 3, in which the larger quantity of gluten was fed, the total amounts being, respectively, in period 1, 3.21 grams; in period 2, 3.26 grams; in period 3, 2.23 grams.

This on its face would indicate that some of the food protein escaped digestion—that is, that there was a so-called depression of digestibility in periods 1 and 2—but the excessive amount of nonprotein nitrogen contained in the feces in period 3 seems to suggest the possibility of an error in the analysis of the latter.

On the whole, Morgen's results seem to negative the hypothesis of any considerable formation of indigestible bacterial protein from the nonprotein of the feed. As regards ammonium salts and asparagin, they agree in this respect with the investigations already summarized in failing to show any increase of either protein nitrogen or pepsin-insoluble nitrogen in the feces. As regards the extracts of various feeding stuffs, the results also agree with the earlier results in showing an increase of the protein nitrogen, but they also strongly support the conclusion that that increase is largely due to the effect of these extracts in stimulating the formation of metabolic products and in part also to the fact that the extract rations contained more pepsin-insoluble nitrogen relatively than did the rations with which they were compared. It should be added, however, that practically all of Morgen's experiments show a greater increase of pepsin-insoluble nitrogen in the feces than can thus be accounted for, although it is not yet clear what interpretation is to be placed upon this fact.

Finally, it should be noted that these negative results neither prove nor disprove the possibility of a formation of *digestible* protein from the nonprotein of the feed.

NUTRITIVE VALUE OF NONPROTEIN FOR HERBIVORA.

NONPROTEIN A SOURCE OF PROTEIN.

In addition to the earlier investigations of Kellner and of Tryniszewski already referred to, experiments have also been made by Andrlik, Velich, and Stanek.[1] Their preliminary trials showed that betain injected into the blood of a dog reappeared quantitatively in the urine, but that when given by the mouth only about one-third thus reappeared, while in a later experiment of the same sort none was found in the urine. They also failed to find betain in the excreta

[1] Velich, Alois, and Stanek, Vladimir. Ueber das Betain in fysiologischchemischer Beziehung. Zweiter Bericht. Zeitschrift für Zuckerindustrie in Böhmen, Jahrgang 29, Heft 4, pp. 205–219. Prag, 1904–1905.

Andrlik, K. and Velich, K. Ueber die Bedeutung der Glutamin- und Asparaginsäure als Nährstoffe. Zeitschrift für Zuckerindustrie in Böhmen, Jahrgang 32, Heft 6, pp. 313–342. Prag. 1907–1908.

of a cow consuming considerable amounts of beet molasses, a substance relatively rich in betain.

After these preliminary trials, an experiment was made on a young wether weighing about 29 kilograms, including trials with betain, glutaminic acid, and aspartic acid. The trials with betain were reported in 1905 and the others in 1908, but all were made in 1904 and apparently constituted a single investigation.

Throughout the experiment the animal received a basal ration of 500 grams hay and 200 grams wheat flour (the latter baked into cakes), with the exception of periods 4 and 5 of the first series, in which one-half of the flour was replaced by starch. This ration approximates to the maintenance requirement of the animal according to the usual standards as regards quantity of feed, but contains considerably more than the minimum of protein. The materials to be tested were simply added to the basal ration.

On the average of all the periods the basal ration contained per day and head:

	Grams.
Total nitrogen	5.05
Protein nitrogen	4.19

In the nonprotein periods the amounts of nitrogen added in the various forms were:

	Grams.
In betain	2.21
In glutaminic acid	1.93
In aspartic acid	2.11

In the first series of trials the periods were short, covering only 5 or 6 days with either one or no intermediate days. In the second series the periods extended over from 14 to 18 days, all of which, however, are included in the averages compared.

Computed in this manner, the gain or loss of nitrogen by the animal was as follows:

Gain or loss of nitrogen—Andrlik, Velich, and Stanek's experiments.

Items.	Gain (+) or loss (−).	Items.	Gain (+) or loss (−).
Series I:	*Grams.*	Series II:	*Grams.*
Period 1, basal ration	+1.14	Period 1, basal ration	+0.97
Period 2, betain	+1.22	Period 2, glutaminic acid	+1.27
Period 3, basal ration	+ .71	Period 3, basal ration	+ .90
Period 4, betain	+ .92	Period 4, aspartic acid	+1.90
Period 5, basal ration	− .42	Period 5, basal ration	+ .97

Omitting periods 4 and 5 of Series I, in which the nitrogen content of the basal ration was reduced, we may compute the following averages apparently showing that the nitrogenous substances added were at least partially utilized in the body of the animal:

	Gram.
For the basal ration	+0.90
For the nonprotein ration	+1.46

The principal evidence regarding the nutritive value of nonprotein for herbivora, however, is derived from later experiments by Kellner on lambs and cows, from the investigations by Morgen et al., whose results as regards the behavior of nonprotein in the digestive tract have just been discussed, and from experiments by the Laboratory for Agricultural Research in Copenhagen.

Kellner [1] experimented on lambs in the belief that growing animals would furnish the most favorable conditions for the utilization of nonprotein. After a vain attempt to use rations containing ammonium acetate or asparagin as the sole source of nitrogen, he succeeded in finding three animals which consumed sufficient amounts of a ration consisting of starch, sugar, ash, asparagin, and ammonium acetate, together with 300 to 400 grams of straw, to enable the experiment to be carried through. This ration contained, of course, a minimum of digestible protein. In a second period the ammonium acetate and asparagin were replaced by wheat gluten nearly equivalent in nitrogen content, and in the case of two of the animals a third period was employed in which ammonium acetate and asparagin equal to that consumed in the first period were added to the ration of the second period. The energy content of the rations was maintained constant by varying the amounts of starch and sugar.

Regarding the pepsin-soluble nitrogen of the straw as the measure of its digestible protein, and counting all the small amount of nitrogenous matter in the starch as digestible, the total content of protein of the basal rations was far below the maintenance requirement of 0.4 kilogram protein per 1,000 kilograms live weight, as appears from the following table: [1]

Digestible protein in basal rations—Kellner's experiments.

Series and periods.	Per head.	Per 1,000 kilograms live weight.
Series I:	*Grams.*	*Kilos.*
Period 1	4.76	0.11
Period 2	4.44	.10
Series II:		
Period 1	5.34	.11
Period 2	4.91	.10
Period 3	4.91	.10

These experiments have already been considered in their bearings upon the fate of the nonproteins in the digestive tract (p. 18). The results as to the production of flesh are shown in the following table of the nitrogen balances per day and head:

[1] Kellner, O., Eisenkolbe, P., Flebbe, R., and Neumann, R. Untersuchungen über den Einfluss einiger nicht-eiweissartiger Stickstoffverbindungen auf den Eiweissumsatz beim Weiderkäuer. Die landwirtschaftlichen Versuchs-Stationem, Band 72, Heft 5–6, pp. 437–458. Berlin, 1910.

[1] The approximate live weights were 44 kilograms in the first series and 48 kilograms in the second series.

Nitrogen balances—Kellner's experiments.

Items.	Period I.		Period II.		Period III.	
	Total nitrogen.	Protein nitrogen.	Total nitrogen.	Protein nitrogen.	Total nitrogen.	Protein nitrogen.
Series I:						
Lamb 1—	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*
In feed	12.19	1.18	11.11	10.19	....	....
In feces	6.73	4.20	4.92	4.25	....	....
In urine	5.37	....	4.26	....	....	....
In total excreta	12.10	....	9.18	....	....	....
Gain	+ .09	....	+1.93	....	....	....
Series II:						
Lamb 2—						
In feed	15.82	1.55	12.86	11.74	26.51	11.62
In feces	5.08	4.37	5.94	4.50	5.51	4.48
In urine	10.16	....	4.28	....	16.17	....
In total excreta	15.24	....	10.22	....	21.68	....
Gain	+ .58	....	+2.64	....	+4.83	....
Lamb 3—						
In feed	15.82	1.55	12.84	11.72	26.51	11.62
In feces	6.68	4.92	6.19	4.33	5.17	4.73
In urine	10.70	....	5.13	....	16.72	....
In total excreta	17.38	....	11.32	....	22.69	....
Gain	−1.56	....	+1.52	....	+3.82	....

Two general conclusions seem to follow from the foregoing results: First, there appears to be clear evidence of a conversion of nonprotein into protein. Although the amount of true protein in the feed in Period I was about one-fourth that required for maintenance, lamb 1 was fully maintained and lamb 2 showed a small gain of nitrogen, while in case of lamb 3 the protein lost from the body plus that supplied in the feed amounts to only 14.66 grams protein per head, equivalent to 0.31 kilogram per 1,000 kilograms live weight, or about three-fourths of the maintenance requirement. Moreover, the nonprotein added in Period III to the ration of Period II caused a notable increase in the storage of protein in the body, either directly or by taking the place of protein previously used for maintenance purposes.

Second, the nonprotein was clearly inferior to protein, for the substitution of the latter for the former in Period II caused a notable gain of protein by the animals in place of approximate maintenance, even although the supply of total nitrogen in the feed was less than in Period I in every case and that of digestible nitrogen less in two cases out of the three.

Kellner's conclusion is that the ammonium salts and asparagin were synthesized by the bacteria of the digestive tract to more complex nitrogenous compounds, possibly even to proteins, and that these compounds were subsequently digested and served in Period I to maintain the protein of the body. The striking difference between the results of Period I and those of Period II he regards as showing

that while nonprotein may thus indirectly perform maintenance functions it is incapable of causing actual growth of protein tissue. The increased gain observed in Period III he explains in the manner previously indicated, viz, that the nonprotein was substituted for protein for maintenance purposes.

But while this explanation is consistent with the experimental results, the existence of such a marked distinction between digested feed protein and digested products of bacterial synthesis appears to the writer unlikely. We can scarcely imagine that this synthesis should result in the production of any simpler compounds than the simple amino acids, while it is more likely to extend at least to the formation of polypeptids if not in part to that of proteins. It is well established, however, that substantially these comparatively simple substances constitute the nitrogenous food of the body, and it is not apparent why their origin through bacterial synthesis should render them any less available than similar substances resulting from enzym cleavage of feed protein. While minor differences may exist, it appears more probable that the limit to the nutritive value of nonproteins to the herbivorous animal is set by the amount which the bacteria are able to synthesize rather than by a difference in the value of the products, and this belief seems to be supported by the investigations at the Hohenheim Experiment Station by Morgen et al. upon milk-producing animals (sheep and goats), about to be considered.

The results of Morgen's investigations have already been discussed in their bearing upon the fate of nonprotein in the digestive tract, but the digestion experiments cited for this purpose formed only part of more extensive investigations, including numerous additional animals, in which the yield and composition of the milk produced were determined.

Perhaps the most striking result of these investigations is the demonstration that nonprotein nitrogen in the form of ammonium salts or asparagin is capable of contributing to the production of milk protein. This conclusion is based upon a comparison of the milk protein with the digestible true protein of the feed, the latter being considered equivalent to the protein nitrogen of feed minus protein nitrogen of feces. At first thought it might seem that only the pepsin-insoluble nitrogen of the feces should be considered in this calculation, since there is good reason to believe that the remainder of the fecal nitrogen is present as metabolic products. But while this is probably true, on the other hand these nitrogenous metabolic products, so far as they are protein in nature (mucus, epithelium, etc.), constitute a loss of protein from the body, and therefore should be taken into account in drawing conclusions from the nitrogen balance.

In the experiments of 1907[1] upon malt sprouts extract, the digestion experiments whose results have already been cited (p. 22) included also determinations of the urinary nitrogen and of the nitrogen in the daily growth of wool. Assuming, on the basis of earlier experiments, a maintenance requirement of 0.4 kilogram digestible protein per 1,000 kilograms live weight (an estimate corresponding almost exactly with Katayama's results[2]), they compute the amount of protein available per day and head for milk production in the periods in which nonprotein was partially substituted for protein as follows:

Protein available for milk production—Morgen's experiments of 1907.

Items.	Sheep 13, period 2.	Sheep 25, period 3.	Goat 28, period 2.	Goat 39, period 3a.
	Grams.	*Grams.*	*Grams.*	*Grams.*
Protein nitrogen available	2.39	3.28	3.00	2.58
Estimated for maintenance	2.88	2.37	2.37	2.56
Remainder	−.49	.91	.63	.02
In growth of wool	.79	.64	....	....
Remainder	−1.28	.27	.63	.02
Loss from body	1.02	1.89	1.53	1.39
Available for milk	−.26	2.16	2.16	1.41
Found in milk	2.29	2.70	3.71	2.83
Deficit	2.55	.54	1.55	1.42
Nonprotein nitrogen in feed	5.60	3.58	5.97	5.96
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Utilization of nonprotein	45.54	15.08	25.96	23.83

In every instance the milk contained more protein than was computed to be available from feed and body protein, while in the extreme case (sheep 13) the feed protein was little more than equal to the milk protein plus wool protein, leaving only 0.33 gram protein nitrogen for maintenance. Evidently the nonprotein must have been utilized either for the production of milk protein or for the maintenance of the body tissues. In the comparison periods in which protein instead of nonprotein was fed, on the other hand, the amount of protein available for milk production, computed in the same manner, was considerably in excess of the protein found in the milk. In general, then, the results of this series of experiments correspond with those obtained in Kellner's experiments on lambs.

The experiments of 1908[3] included two trials on malt sprouts extract, one each upon grass extract and mangel extract, eight upon ammonium acetate, one upon ammonium tartrate, and two upon

[1] Morgen, A., Berger, C., and Westhausser, F. Weitere Untersuchungen über den Einfluss der nicht-eiweissartigen Stickstoffverbindungen der Futtermittel auf die Milchproduktion. Die landwirtschaftlichen Versuchs-Stationen, Jahrgang 68, Heft 5–6, pp. 333–432. Berlin, 1908.

[2] Katayama, T. Über das Eiweiss-Minimum für ausgewachsene Hammel. Die landwirtschaftlichen Versuchs-Stationen, Band 69, Heft 5–6, pp. 321–341. Berlin, 1908.

[3] Morgen, A., Beger, C., and Westhausser, F. Weitere Untersuchungen über die Verwertung der nicht-eiweissartigen Stickstoffverbindungen der Futtermittel sowie der Ammonsalze durch das milchgebende Tier unter besonderer Berücksichtigung der stickstoffhaltigen Stoffwechselprodukte. Die landwirtschaftlichen Versuchs-Stationen, Band 71, Heft 1–3, pp. 1–170. Berlin, 1909.

asparagin, in all of which the nitrogen balance was determined.[1] Computed as before, the average results per day and head for each nonprotein material were as follows:

Protein available for milk production—Morgen's experiments of 1908.

Items.	Ammonium salts.	Asparagin.	Extract of malt sprouts.	Extract of grass.	Extract of mangels.
	Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*
Protein nitrogen available	5.69	5.53	3.93	4.92	6.88
Estimated for maintenance	2.57	2.50	3.14	2.62	2.50
Remainder	3.12	3.03	.79	2.30	4.38
In growth of wool	.47	.54	.70	.48	....
Remainder	2.65	2.49	.09	1.82	4.38
Loss from body	.18	− .01	2.28	.75	− .49
Available for milk	2.83	2.48	2.37	2.57	3.89
Found in milk	4.21	3.92	3.95	2.17	3.51
Deficit	1.38	1.44	1.58	....	....
Nonprotein nitrogen in feed	6.23	6.19	7.98	....	....
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Utilization of nonprotein	22.15	23.26	19.80	....	....

All the experiments, except the single trials upon extracts of grass and of mangels, showed a deficit of available protein as compared with the amount found in the milk, while, as in 1907, the comparison periods in which protein was fed showed a surplus. The exceptional results with the two extracts may, perhaps, be ascribed to the fact that in these trials a smaller proportion of the digestible protein was replaced by nonprotein than in the other cases (about 21 per cent as compared with 33 to 38 per cent), although the replacement was carried as far as in the experiments with sheep in 1907.

The experiments of 1909 [2] showed even more striking results in the four cases in which about 63 per cent of the digestible true protein was replaced by ammonium acetate. The individual results, per day and head, computed as in the preceding cases, were:

Protein available for milk production—Morgen's experiments of 1909.

Items.	Sheep 48, period 2.	Sheep 49, period 2.	Sheep 50, period 2.	Sheep 56, period 3.
	Grams.	*Grams.*	*Grams.*	*Grams.*
Protein nitrogen available	2.89	2.73	2.70	2.70
Estimated for maintenance and wool	3.55	3.67	3.35	3.33
Remainder	− .66	− .94	− .65	− .63
Loss from body	− .16	.24	− .45	− .27
Available for milk	− .82	− .70	−1.10	− .90
Found in milk	4.15	3.61	3.55	3.19
Total deficit	4.97	4.31	4.65	4.09
Nonprotein nitrogen in feed	11.65	11.65	10.35	10.35
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Utilization of nonprotein	42.7	37.0	44.9	39.5

[1] The results as regards digestibility are tabulated on page 24.

[2] Morgen, A., Beger, C., and Westhausser, F. Untersuchungen über die Verwertung der Ammonsalze und der nicht-eiweissartigen Stickstoffverbindungen der Futtermittel für die Lebenserhaltung und Milchbildung, sowie über die Frage ob aus diesen Stoffen unverdauliches Eiweiss gebildet wird. Die landwirtschaftlichen Versuchs-Stationen, Band 73, Heft 4–5, pp. 285–396. Berlin, 1910.

The only conclusion which can be drawn from these results is that the ammonium acetate served as a source of milk protein. Any error in the estimate of the maintenance requirement is without significance, since, even if we disregard the slight gain of protein by the body in three cases, the total amount of protein contained in the rations (plus that supplied from the body by sheep 49) is notably less than the amount actually found in the milk. The obvious interpretation of these results is that some of the ammonium acetate was synthesized to protein by means of bacteria and subsequently digested.

On the other hand, the seven trials in which ammonium acetate was added to a basal ration containing a moderate amount of digestible protein, instead of being substituted for the latter, yielded no such results, the true protein present being more than sufficient for all purposes. The same thing was likewise true of the trials in which extracts of grass and of dried beet pulp were added to the basal ration.

A similar result has also been reported by Kellner [1] in an experiment on a milch cow. The protein content of the ration was gradually diminished until the excess over the maintenance requirement was practically equal to the protein of the milk, and then a part of the remaining protein was replaced by ammonium acetate and starch, the total ration in both cases being sufficient to cause some gain of body fat. The results as regards nitrogen were as follows:

Protein available for milk production—Kellner's experiments.

Items.	Period VI, with ammonium acetate.	Period VII, without ammonium acetate.
	Grams.	*Grams.*
Protein nitrogen, digested	22.23	53.83
Estimated for maintenance	28.98	28.98
Remainder	— 6.75	24.85
Gain or loss by body	— 1.96	+ 1.86
Available for milk	— 4.79	22.99
In milk	53.37	55.79
Deficit	58.16	32.80
Nonprotein nitrogen in feed	62.03	34.70
	Per cent.	*Per cent.*
Utilization of nonprotein	93.76	94.52

Even in Period VII there appears to have been a slight formation of milk from the nonprotein of the feed, while in Period VI the phenomenon is even more striking than in Morgen's experiments. Kellner, it is true, has subsequently [2] expressed the opinion that his results do not prove such a utilization of nonprotein, since it is uncertain

[1] Kellner, Oskar. Die Ernährung der Landwirtschaften Nutztiere, 4th ed., p. 539.

[2] Kellner, Oskar. Ibid., 5th ed., p. 549.

how much of the indigestible protein found in the feces may have been bacterial protein derived from the nonprotein of the feed. As has been shown in the foregoing pages, however, the evidence is strongly against such an action in the digestive tract, and it would seem that Kellner's results must rank with Morgen's as showing a possible utilization of ammonia for the production of milk protein.

Experiments upon the minimum protein requirements of dairy cows reported from the Laboratory for Agricultural Research in Copenhagen in 1906–7 [1] afford confirmation of Morgen's results. In these experiments the cottonseed cake contained in the initial ration was gradually withdrawn, while the amount of fodder beets was increased so as to maintain unchanged the number of feed units in the ration computed according to Fjord's system. The protein nitrogen of the ration was thus largely reduced while the amount of nonprotein shows but slight differences. The experiments, therefore, show little as to the behavior of nonprotein in the digestive tract or as to its nutritive value as compared with that of protein. Like Morgen's experiments of 1907 and 1908, however, they appear to show that the nonprotein of the rations must have served at least for the maintenance of the protein tissues of the body, while in some instances the assumption of a small formation of milk protein from nonprotein seems necessary. The following table, compiled from the Sixtieth Report, shows the results of those of the experiments in which the smaller amounts of true protein were consumed. On the average of all these trials, the nonprotein nitrogen constituted about 23 per cent of the total nitrogen. The table shows that in general the protein available from the feed, plus that contributed by the body protein, is approximately equal to the milk protein, so that the maintenance function at least must have been supported by the nonprotein.

Results of Copenhagen experiments—Sixtieth Report.

Items.	Protein nitrogen digested.[2]	Gain of nitrogen by body.	Protein nitrogen available for milk production.	Nitrogen in milk.	Surplus for maintenance.	Estimated maintenance requirement.	Deficit of protein nitrogen.	Nonprotein nitrogen in feed.	Percentage utilization of nonprotein nitrogen.
Cow No. 10:	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Per ct.*	*Per ct.*
Period 4	47	−16	63	63	0	32	32	35	91.4
Period 5	44	−12	56	60	−4	32	36	36	100.0
Period 6	57	− 2	59	62	−3	32	35	39	89.7
Cow No. 23:									
Period 4	47	− 9	56	59	−3	35	38	34	111.8
Period 5	47	−15	62	57	5	35	30	36	83.3

[1] Denmark—Beretning fra den Kgl. Veterinaer of Landbohojskoles Laboratorium for landokonomiske Forsog. 60de, 1906, and 63de, 1907, Kobenhavn. Translated by Mallevre, Société de l'Alimentation Rationale du Bétail. Compte Rendu de 11ème et 12ème Congrès.

[2] Protein nitrogen of feed minus protein nitrogen of feces.

Results of Copenhagen experiments—Sixtieth Report—Continued.

Items.	Protein nitrogen digested.	Gain of nitrogen by body.	Protein nitrogen available for milk production.	Nitrogen in milk.	Surplus for maintenance.	Estimated maintenance requirement.	Deficit of protein nitrogen.	Nonprotein nitrogen in feed.	Percentage utilization of nonprotein nitrogen.
Cow No. 53:	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Per ct.*	*Per ct.*
Period 4	53	− 2	55	56	−1	30	31	34	91.2
Period 5	28	−13	41	45	−4	30	34	39	87.2
Period 6	50	+ 3	47	49	−2	30	32	41	78.1
Cow No. 68:									
Period 4	57	− 5	62	63	−1	32	33	35	94.3
Period 5	44	−16	60	58	2	32	30	36	83.3
Period 14	48	− 2	50	50	0	32	32	33	97.0
Cow No. 58:									
Period 4	51	− 1	52	52	0	32	32	34	94.1
Period 5	27	−13	40	45	−5	32	37	37	100.0
Period 6	55	+ 6	49	47	2	32	30	38	78.9
Total	655	−97	752	766	−14	448	462	507	91.1

In the experiments recorded in the Sixty-third Report the fact is taken into account that a portion of the nitrogen of the beets existed in the form of nitrates, which, as is well known, yield free nitrogen in the digestive tract. For this reason the loss of protein from the body is somewhat greater than that which would be computed from the income and outgo of nitrogen in the usual way. When the results of the experiments are computed after the ordinary method, they show in several cases a distinct deficit of protein; that is, the protein available from the food plus that contributed by the body is distinctly less than the protein of the milk. When the correction for nitrates is applied, however, these differences either disappear or become almost negligible, as is shown by the following table:

Results of Copenhagen experiments—Sixty-third Report.

Items.	Protein nitrogen digested.	Gain of nitrogen by body.	Protein nitrogen available for milk production.	Nitrogen in milk.	Surplus for maintenance.	Estimated maintenance requirement.	Deficit of protein nitrogen.	Nonprotein nitrogen in feed.	Percentage utilization of nonprotein nitrogen.
Cow No. 68:	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Per ct.*
Period 3	57	−16	73	65	8	30	22	35	62.9
Period 4	33	−26	59	59	....	30	30	35	85.7
Period 5	30	−23	53	58	−5	30	35	36	97.2
Period 6	53	− 4	57	59	−2	30	32	39	82.1
Cow No. 125:									
Period 4	69	−17	86	83	3	33	30	55	54.6
Period 5	69	−12	81	83	−2	33	35	54	64.8
Period 6	87	+ 4	83	79	4	33	29	55	52.7
Period 7	94	+ 7	87	79	8	33	25	54	46.3

EFFECT OF NONPROTEIN ON TOTAL PRODUCTION.

But while Morgen's results on milking animals differ from Kellner's on lambs in this one point, their results are entirely in accord in showing that the nutritive value of nonprotein is much inferior to that of protein. This becomes apparent as soon as we turn from a study of the digestion trials and nitrogen balances to a consideration of the actual yield of milk and its constituents on the various rations. The preliminary experiments of 1906 [1] showed a marked decrease in the milk production when nonprotein was substituted for protein, and the more elaborate experiments of the following years only confirmed this result.

The experiments were made after the so-called "period system," the natural decrease in the milk yield with advancing lactation being estimated by a comparison between an initial and a final period on identical rations. In comparing his results Morgen simply adds the correction thus computed for each period to the observed yield, and thus computes what the yield would have been had there been no depression due to advancing lactation, and these corrected numbers constitute the basis for comparing the effects of the rations. It may easily be shown, however, that this method of computation is incorrect and tends to reduce the real effects of the changes in the rations. For example, in one experiment the average daily yields of milk solids were:

	Grams.
Period 1, protein ration	105.70
Period 2, malt sprouts extract ration	36.62
Period 3, protein ration	36.25

From the middle of period 1 to the middle of period 3 was 78 days, so that the average daily falling off in the yield of milk solids was 0.8904 gram, and for the 40.5 days between period 1 and period 2 amounted to 36.08 grams. Morgen therefore makes the following comparison of the yields of milk solids:

	Grams.	Grams.
Period 1		105.70
Period 2:		
Observed	36.62	
Correction	36.08	
Computed		72.70
Period 3:		
Observed	36.25	
Correction	69.45	
Computed		105.70

[1] Morgen, A., Beger, C., and Westhausser, F. Untersuchungen über den Einfluss der nicht-eiweissartigen Stickstoffverbindungen der Futtermittel auf die Milchproduktion. Die landwirtschaftlichen Versuchs-Stationen, Band 65, Heft 5–6, pp. 313–440. Berlin, 1906–7.

and computes that the effect of the extract ration was $72.70 \div 105.70 = 68.8$ per cent of that of the protein ration. The assumption underlying such a correction for the advance of lactation, however, is that if the feed had been unchanged the falling off in yield would have been proportional to the time. In this case, therefore, the falling off up to the middle of period 2 would have been, as computed, 36.08 grams, and consequently the yield of milk solids on an unchanged ration would have been $105.70 - 36.08 = 69.62$ grams, so that we may make the following comparison:

Periods.	Observed yield.	Computed yield.	Observed in percentage of computed.
	Grams.	*Grams.*	*Per cent.*
1	105.70		
2	36.62	69.62	52.61
3	36.25		

Morgen's method of computation, in other words, adds the same correction to two unequal quantities; and therefore, while the difference between the two is unaffected, the ratio between them is distorted in favor of the smaller number.

In the experiments of 1907 the basal rations contained approximately 2.5 kilograms of digestible protein per 1,000 kilograms live weight, of which approximately 0.9 kilogram was replaced by the nonprotein of malt sprouts extract. The following table shows the production of milk solids and of milk protein expressed as a percentage of the amount which it is computed would have been produced had the protein ration been continued unchanged, the computation being made in the manner just indicated.[1]

[1] In certain of the experiments only a three-fourths ration could be fed in the nonprotein periods, and corresponding periods were introduced in which three-fourths of the normal protein ration was fed. In these cases the results have been computed on the assumption that the rate of decrease in milk production would have been the same as was actually observed between the two full protein rations. For example, in case of sheep 13 (loc. cit., p. 402) the following results were obtained:

	Milk solids.	Milk protein.
	Per cent.	*Per cent.*
Correction (period 3–2)	22.85	1.02
Observed yield in period 3 on three-fourths protein ration	46.47	2.54
Computed yield in period 2 on three-fourths protein ration	69.32	3.56
Observed yield in period 2	40.78	2.29
Observed in percentage of computed	58.84	64.33

Yield in per cent of yield in protein periods—Morgen's experiments of 1907.

Items.	Milk solids.	Milk protein.
Malt sprouts extract periods:	*Per cent.*	*Per cent.*
Sheep 13	58.84	64.33
Sheep 22	84.38	83.58
Sheep 25	75.38	74.62
Sheep 27	58.07	54.72
Sheep 27 (grass extract)	75.48	65.57
Sheep 30	71.52	71.16
Sheep 32	69.00	67.76
Sheep 34	61.44	59.76
Average of percentages	69.26	67.69
Carbohydrate periods:		
Sheep 22	74.49	73.71
Sheep 27	59.12	55.56
Sheep 30	83.58	83.59
Sheep 32	61.62	57.99
Sheep 34	52.12	52.49
Average of percentages	66.19	64.67

In general, the relative yield in the malt sprouts extract periods is somewhat greater than that obtained from a corresponding quantity of carbohydrates, although there are individual exceptions. Apparently the nonprotein, while greatly inferior to protein, had a somewhat greater nutritive value than the carbohydrates.

In the experiments of 1908 the basal ration contained 2.3 kilograms digestible protein per thousand live weight. In the various periods either 28 per cent or 44 per cent of this protein was replaced by nonprotein derived from various sources, viz, from malt sprouts extract, grass extract, mangel extract, ammonium salts, and asparagin, while six carbohydrate periods were also introduced. The relative yields, computed as in the preceding case, using the yield in periods 1 and 5 on the protein ration as the basis of computation, were as follows:

Yield in per cent of yield in protein periods—Morgen's experiments of 1908.

Items.	Percentage of protein replaced.	Milk solids.	Milk protein.
Malt sprouts extract:		*Per cent.*	*Per cent.*
Sheep 13	44	52.61	62.40
Sheep 22	44	79.67	79.97
Sheep 27	28	67.44	70.55
Sheep 30	44	55.91	55.56
Sheep 32	44	63.71	64.63
Sheep 33	44	48.57	48.70
Sheep 42	28	51.92	52.74
Goat 53	28	82.26	85.71
Average of percentages	....	62.76	65.03
Grass extract:			
Sheep 49	28	53.08	46.77
Goat 31	28	84.39	82.87
Goat 38	28	73.72	62.86
Goat 41	28	54.51	48.28
Goat 45	28	62.92	53.62
Goat 52	28	69.05	57.49
Goat 53	28	85.71	89.18
Average of percentages	....	69.05	63.01

Yield in per cent of yield in protein period—Morgen's experiments of 1908—Continued.

Items.	Percentage of protein replaced.	Milk solids.	Milk proteins.
Mangel extract:		*Per cent.*	*Per cent.*
Sheep 34	28	72.96	68.21
Sheep 42	28	59.08	51.74
Goat 28	28	89.96	84.79
Average of percentages	....	74.00	68.25
Ammonium salts:			
Sheep 34, acetate	28	102.35	93.75
Sheep 35, acetate	44	93.11	83.03
Sheep 35, acetate as drink	44	107.30	90.33
Sheep 37, acetate	44	80.01	75.75
Sheep 37, acetate as drink	44	78.12	74.25
Sheep 48, tartrate	44	62.28	66.91
Sheep 48, acetate	44	86.32	79.50
Sheep 49, acetate	44	72.97	72.70
Sheep 50, acetate	44	69.99	73.77
Goat 28, acetate	28	94.69	86.27
Goat 40, phosphate	44	81.13	85.11
Goat 41, acetate	28	87.34	82.96
Goat 45, acetate	28	66.13	70.51
Average of percentages	....	83.21	79.60
Asparagin:			
Sheep 35	44	80.50	81.82
Sheep 50	44	63.44	61.28
Average of percentages	....	71.97	71.55
Average of ammonium salts and asparagin	....	81.72	78.54
Carbohydrates:			
Sheep 30	44	54.53	52.74
Sheep 33	44	56.96	57.33
Average of percentages	....	55.75	55.04
Sheep 31	28	93.63	95.47
Sheep 38	28	83.78	82.06
Sheep 40	44	95.24	94.70
Sheep 52	28	72.65	70.35
Average of percentages	....	86.33	85.65
Protein withdrawn:			
Sheep 32	44	63.26	64.25
Averages:			
Malt sprouts extract	....	62.76	65.03
Grass extract	....	69.05	63.01
Mangel extract	....	74.00	68.25
Ammonium salts and asparagin	....	81.72	78.54
Carbohydrates—			
Sheep	....	55.75	55.04
Goats	....	86.33	85.65

The results upon the individual animals were more or less variable, a fact probably due in part to differences in the order of the various periods and perhaps to individual differences. In general, the extracts gave results fully as low as in the experiments of 1907, but also somewhat higher than those obtained in the carbohydrate periods with sheep. The results with goats in the latter periods seem exceptional. On the other hand, the relative yield upon ammonium salts and asparagin was notably greater than on the plant extracts, although falling materially short of that obtained with protein.

It should be noted that the low value of the extracts as compared with ammonium salts may be due to a small extent to a specific effect

of these materials on the milk production, as appears from the results of the next year.

In the experiments of 1909 the basal ration contained 2.4 kilograms of digestible protein per thousand live weight. In eight trials 63 per cent of this was replaced by ammonium acetate containing an equal amount of nitrogen. In the other trials ammonium acetate and extracts of grass, malt sprouts, and dried beet pulp were simply added to the basal ration. The results, computed as before, are contained in the following table:

Yield in per cent of yield in protein periods—Morgen's experiments of 1909.

Items.	Milk solids.	Milk protein.
Ammonium acetate—substitution experiments:	*Per cent.*	*Per cent.*
Sheep 32	52.89	54.63
Sheep 48	60.68	58.13
Sheep 49	57.68	56.94
Sheep 50	64.85	61.42
Sheep 56	58.96	59.30
Sheep 57	73.50	75.26
Sheep 61	59.69	64.63
[Goat 51]	[88.37]	[77.58]
Average of percentages (omitting goat 51)	61.04	61.47
Ammonium acetate—addition experiments:		
Sheep 32	85.49	91.08
Sheep 48	79.44	74.38
Sheep 49	93.57	91.62
Sheep 50	93.06	89.34
Sheep 56	108.44	102.23
Sheep 57 (period 3)	95.39	99.17
Sheep 57 (period 4)	107.55	105.21
Sheep 61	94.29	92.46
[Goat 51]	[144.25]	[142.20]
Average of percentages (omitting goat 51)	94.15	93.19
Grass extract—addition experiments:		
Sheep 35	85.33	82.96
Sheep 42	93.77	85.91
Goat 45	93.57	90.07
Average of percentages	90.89	86.31
Malt sprouts extract—addition experiments:		
Goat 38	93.17	91.25
Goat 40	91.50	85.51
Goat 52	92.46	87.07
Average of percentages	92.38	87.94
Beet-pulp extract—addition experiments:		
Sheep 35	86.87	84.84
Sheep 42	80.91	73.21
Goat 38	91.66	89.45
Goat 40	95.51	86.23
Goat 45 (period 3)	77.60	75.64
Goat 45 (period 4)	77.41	73.55
Goat 52	84.56	83.78
Average of percentages	84.93	80.96
Averages:		
Ammonium acetate—substitution experiments	61.04	61.47
Ammonium acetate—addition experiments	94.15	93.19
Grass extract—addition experiments	90.89	86.31
Malt sprouts extract—addition experiments	92.38	87.94
Beet-pulp extract—addition experiments	84.93	80.96

In the substitution experiments with ammonium acetate, the milk yield fell off much more than in 1908, obviously because the substitu-

tion was carried much further. On the other hand, the addition of ammonium salts to the basal ration produced practically no effect upon the yield, the small differences noted being scarcely significant. The addition of the various extracts seems to have caused some decrease in the yield, which, as already suggested, may indicate a specific effect, but one which is not great enough to account for the falling off noted in the substitution experiments of the previous year.

The foregoing results fully confirm the conclusions drawn from a study of the nitrogen balances, and show that the ammonium salts, asparagin, and plant extracts all seem to have enabled the animals to produce a greater amount of milk than did a corresponding amount of carbohydrates. With the plant extracts this difference is comparatively slight, but with ammonium acetate and asparagin it is quite decided. It would appear as if these latter readily soluble materials are more easily converted into protein by the bacteria of the digestive tract than are the diverse nonprotein substances of plant extracts.

On the other hand, the amount of nonprotein nitrogen thus rendered available was relatively small. The organism reacted to the replacement of protein by ammonium salts chiefly by diminishing the amount of milk produced, while under these conditions it was able to utilize that portion of the nonprotein rendered available by bacterial action. When, however, the protein supply was reasonably abundant, the amount which may have been formed from the added nonprotein under those circumstances produced no perceptible effect upon the milk yield.

These relations are perhaps most apparent in the case of the four digestion experiments of 1909 in which the protein of the basal ration was replaced by ammonium acetate (p. 34). If we compute in the same manner as in the foregoing cases what the amount of nitrogen in the milk yield would have been had the protein ration been continued, we get the following results:

Influence of feed protein on yield of milk protein—Morgen's experiments of 1909.

Items.	Yield of milk protein.	
	Observed for nonprotein ration.	Computed for protein ration.
	Grams.	*Grams.*
Yield of nitrogen in milk:		
Sheep 48	4.15	7.14
Sheep 49	3.61	6.34
Sheep 50	3.55	5.78
Sheep 56	3.19	5.38
Average	3.63	6.16
Average available protein nitrogen of feed	2.76	13.28

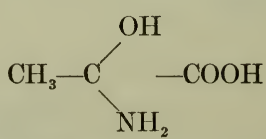
On the protein rations the supply of food protein was sufficient to cover the demand for maintenance, the gain of protein by the body, and the production of protein in the milk, and to leave an average surplus of 3.48 grams nitrogen. When, however, the available protein supply was reduced to the low figure of 2.76 grams nitrogen, or 21 per cent of the previous amount, the yield by the animals was reduced in the case of the milk solids by 39 per cent and in the case of the milk protein by 41 per cent. This large falling off makes it evident that the protein supply was the limiting factor of milk production in these periods. Under these conditions of limited protein supply, however, the nonprotein nitrogen of the feed was utilized to a certain extent as is shown by the nitrogen balances, so that the falling off in the yield of milk was not proportional to the reduction in the protein supply.

DIRECT UTILIZATION OF AMMONIUM SALTS.

It will not have escaped notice that the evidence of the utilization of nonprotein by means of the formation of bacterial protein which is furnished by the foregoing experiments is indirect. Since it has not been satisfactorily shown that carnivora or omnivora can utilize nonprotein as a source of protein, it is concluded that the opposite results with herbivora can not be ascribed to a synthetic production of protein in the processes of metabolism, but must be due to some other cause, the formation of bacterial protein appearing the most probable one.

Moreover, the most decided nutritive effect is obtained with ammonium salts, i. e., precisely those compounds which seem least likely to be subject to metabolic synthesis. In this connection, however, attention should be called to two recent papers.

Knoop[1] claims to have established the theoretical possibility of a formation of amino acids in the body of the dog from ammonia and nonnitrogenous substances. According to him, the deamidization of the amino acids resulting from protein cleavage in metabolism is a process of oxidation, giving rise to the corresponding keto and oxy acids and ammonia. This change he regards as a reversible reaction and assumes the formation of hypothetical intermediate products of the type



from which either ammonia or water may be split off according to the direction of the reaction.

[1] Knoop, F. Über den physiologischen Abbau der Säuren und die Synthese einiger Aminosäuren im Tierkorper. Zeitschrift für physiologische Chemie, Band 67, Heft 6, pp. 289–502. Strassburg, 1910. See p. 489.

It does not appear that Knoop actually experimented with ammonium salts, but Embden and Schmitz[1] in perfusion experiments on the liver have observed the formation of tyrosin, phenylalanin, and alanin, and probably of leucin, when the ammonium salts of corresponding acids were added to the perfused blood. The yield of alanin was especially large from pyruvic acid and less so from lactic acid, which corresponds with Knoop's view. When a liver rich in glycogen was perfused with blood containing ammonium chlorid, alanin was also obtained. This fact the authors explain as due to a formation of lactic acid from liver carbohydrate, and regard it as showing the possibility of the formation of an amino acid from ammonia and a carbohydrate. Even if the foregoing results are confirmed by further investigation, their significance for questions of nutrition may perhaps be questioned; but, nevertheless, the possibility of a synthesis of ammonia to amino acids, and thence to protein, by higher animals as well as by lower, should not be lost sight of.

RÉSUMÉ.

The results recorded in the foregoing pages may be briefly summarized as follows:

1. Amino acids and amids, which ordinarily constitute the larger part of the nonprotein of vegetable substances, are katabolized in the animal body, their nitrogen appearing in the urine.

2. In carnivora and omnivora neither the single substances of the foregoing groups nor the mixtures of them contained in plant extracts have been shown to be capable of performing the functions of protein.

3. In ruminants a conversion of nonprotein into protein appears to be effected by the micro-organisms of the digestive tract. The extent of this conversion appears to be relatively greater in the case of ammonium salts and asparagin than in that of vegetable extracts.

4. The protein formed thus from nonprotein seems to be digested subsequently. The apparent formation of indigestible protein observed by some investigators appears to be due to an increase in the metabolic products contained in the feces, caused by a specific action of the extracts upon the digestive tract.

5. By means of its conversion into bacterial protein, the nonprotein of feeds may serve indirectly for maintenance and also as a source of protein for milk, and probably for growth, in rations deficient in protein.

6. The limiting factor in the indirect utilization of the nonprotein of the feed appears to be the extent to which it can be converted into protein in the digestive tract rather than any inferior nutritive

[1] Embden, Gustav, and Schmitz, Ernst. Über synthetische Bildung von Aminosäuren in der Leber. Biochemische Zeitschrift, Band 29, Heft 6, pp. 423–428. Berlin, 1910.

value of the protein thus formed as compared with that originally present in the feed.

7. The nonproteins are much inferior to the proteins in nutritive value for productive feeding. The prime effect of a substitution of nonproteins for proteins in the ration is a very marked falling off in the production. The indirect utilization of nonprotein simply serves to prevent this decrease from becoming as great as it otherwise would, and so in case of need to compensate partially for a deficiency of protein. On the other hand, with a reasonable supply of digestible protein the addition of nonprotein usually fails to increase the production of nitrogenous matter.

8. Recent experiments raise the question of the possibility of a direct utilization of ammonia as a source of protein by the higher animals.

CONCLUSIONS.

If the foregoing summary may be regarded as expressing with substantial accuracy the present state of our knowledge regarding the behavior of nonprotein in the animal body, what conclusions can be drawn from the facts there set forth as to the value to be assigned to this group in the computation of rations for farm animals?

VALUE FOR MAINTENANCE OF PROTEIN TISSUES.

It appears to be well established that nonprotein may be of equal value with protein for the maintenance of the protein tissues of the body, so far at least as this can be determined from the nitrogen balance.

Kellner's experiments on lambs (pp. 30–32) show qualitatively that ammonium salts and asparagin may perform the functions of protein in this respect, but they were present in excess and but a comparatively small proportion of them was utilized. In the experiments on cows reported by the Danish investigators (pp. 36–37) little or no protein was left after the demands of milk production were met, and the maintenance function must have been supported almost wholly by the nonprotein. Even assuming a minimum value for the maintenance requirement, there are a number of cases in which nearly or quite 100 per cent of the nonprotein appears to have been thus utilized, while in Kellner's experiment (p. 35) the available feed protein did not even equal that produced in the milk. In these cases at least, it seems necessary to conclude that a unit of nonprotein nitrogen in the ration was of equal value with a unit of protein nitrogen. Morgen's experimental results (pp. 32–35) occupy in this respect an intermediate position. They show that the nonprotein must have served for either maintenance or production, but a relatively small proportion of it (15 to 41 per cent) was utilized in this way.

VALUE FOR PRODUCTION.

With the exception of Kellner's and Morgen's experiments with ammonium acetate, there is as yet no positive evidence that non-protein can replace protein for productive purposes, and ammonium salts do not occur in ordinary feeding stuffs in any considerable amount. In Morgen's experiments with plant extracts the supply of protein was more than sufficient in all cases to meet the demands of the diminished milk production. In the Danish experiments, with a relatively heavier milk production in the low protein periods, the limit was more nearly reached, but there can hardly be said to have been a significant deficit of protein in any instance.

As was stated on pages 32 and 35, it appears to the writer probable that the limiting factor in these cases was the extent to which the bacterial synthesis of protein was carried, rather than an inferiority in the nutritive value of the product. But however this may be, the practical result, from the standpoint of the computation of rations, is as if the nonprotein contained in rations such as are ordinarily fed may serve for maintenance but not for production. Whether a more extensive substitution of this type of nonprotein in place of protein would yield a different result can of course be decided only by experiment. It may be remarked, however, that the proportion of non-protein to protein in the recorded experiments appears to be as great as it is likely to be in any ordinary ration, and, pending further evidence, it would seem to be the part of safety to consider that ordinarily not enough of the nonprotein is converted into protein (by bacterial action or otherwise) to make it of any material significance for the production of milk protein (and probably, therefore, of protein tissue).

THE COMPUTATION OF RATIONS.

If, however, the nonprotein is to be regarded as of full value for maintenance but as practically valueless for production, an undesirable complication is introduced into the computation of rations. The value of a feeding stuff (as regards protein) in a maintenance ration would be measured by its total nitrogen ("crude protein"), while the corresponding value of the same feeding stuff for productive purposes would be measured by its protein nitrogen.

But a considerable part of every productive ration serves for the maintenance of the animal. A part of the protein requirement, therefore, might be met indifferently by either protein or nonprotein, while for the remainder only protein would serve. For example, suppose a dairy cow to require per day 0.5 pound protein for maintenance and 1.75 pounds for the production of 35 pounds of average milk. She must be supplied with a ration containing a total of 2.25

pounds of digestible nitrogenous matter, in which, however, the nonprotein may vary from 0 to 0.5 pound, but may not exceed the latter limit. If, then, the "crude" protein ($N \times 6.25$) of the ration is made the basis of the computation and a ration is formulated supplying the necessary 2.15 pounds of digestible nitrogenous matter, a supplementary calculation would be required to determine whether or not the limit for nonprotein has been exceeded. Such a calculation, while it might signify little to the expert, would constitute an additional difficulty in the way of teaching the computation of rations to the practical farmer, who usually finds the subject sufficiently unfamiliar and complicated even when presented in the simplest possible way.

It is to be remarked in the first place that, so far as appears from the results cited in the foregoing pages, the nonprotein of feeding stuffs is available for the maintenance of ruminants only, while in the case of swine and probably of horses only the protein can be used for this purpose. In the case of the two latter species, therefore, it is evident that the digestible protein should be made the basis of the computation. Ultimately, of course, we should have separate tables of feed values for these animals, since their digestive capacity is in some respects materially different from that of ruminants. For the present, or as long as we continue to use a single table for all species of domestic animals, it seems undesirable to complicate the calculation in the case of ruminants by introducing the nonprotein into the calculation. Pending further investigation, therefore, it would appear to be the wisest course to continue to use ordinarily the digestible true protein as the basis of computing rations, ignoring the nonprotein. While doing this, however, it should be appreciated that rations thus computed will be likely to be unnecessarily high in protein, especially if composed largely of feeding stuffs rich in nonprotein, such as roots, silage, and green forage. This will be particularly the case when the protein requirement for maintenance constitutes a large proportion of the total protein requirement, as, for example, in working horses or in mature fattening animals; while, on the other hand, the error will be least with growing stock or good dairy cows where the productive quota is large as compared with the maintenance requirement.

If it is desired to make the computation more accurate in the case of ruminants, so as to avoid any excess of the relatively costly protein, it would appear that this end might be most simply reached by the method about to be suggested. This consists in formulating separately the protein requirement for maintenance and for productive purposes, computing a ration which shall supply sufficient true protein to meet the requirement for production, and then computing whether this ration contains sufficient nonprotein to cover the maintenance

requirement.[1] Suppose, for example, it is desired to compute a ration for a 1,000-pound cow producing daily 35 pounds of average milk. Using for illustration the requirements formulated by the writer,[2] the ration of the animal should contain, for milk, 1.6 pounds protein and 9.6 therms energy value; for maintenance, 0.5 pound protein or nonprotein, and 6 therms energy value.

Disregarding in the first instance the nonprotein requirement, we may compute the following ration which supplies the necessary amounts of true protein and of energy:

Ration.	Dry matter.	Digestible protein.	Nonprotein.	Energy.
	Pounds.	*Pounds.*	*Pound.*	*Therms.*
Silage, 40 pounds	10.2	0.26	0.21	6.64
Clover hay, 15 pounds	12.7	.80	.30	5.21
Corn chop, 5 pounds	4.2	.22	.02	3.60
Cottonseed meal, 1 pound	.9	.35	.02	.84
	28.0	1.63	.55	16.29

A supplementary calculation shows that the foregoing ration would also contain 0.55 pound nonprotein, the amount supplied by each feeding stuff being included for convenience in the foregoing table. The ration as computed thus proves to contain a slight excess of nonprotein over the estimated maintenance requirement, and may therefore be regarded as adequate, while if the total requirement for nitrogenous matter had been supplied by true protein it would have been necessary to use at least $1\frac{1}{2}$ pounds more of cottonseed meal in the ration. While this method of computation adds slightly to the labor of computing rations for ruminants, it has the advantage of tending to economy in the use of protein concentrates, a thing which appears desirable since these are usually the expensive ingredients of the ration and since recent investigation indicates strongly that the protein requirements of animals have been more or less exaggerated in the current feeding standards.

[1] For this purpose it would of course be necessary that the table used should show the percentage of nonprotein in the feeding stuffs in question.

[2] Armsby, Henry P. The computation of rations for farm animals by the use of energy values. U. S. Department of Agriculture, Farmers' Bulletin 346. Washington, 1909. See p. 19.

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